

Reform, not much improvement

The British educational system, already punch-drunk from too much change, is due for another upheaval, but the government's new education bill is a poor and even dangerous recipe.

MR Kenneth Baker's Education Reform Bill, published last Friday, is misnamed. It will probably serve its intended purpose of standing the British educational system on its head. It will also keep the British parliament wrangling for most of the coming parliamentary year. But, with one important exception, the document has nothing to do with education, only administration. And (with two or three exceptions) the changes advocated by the bill are not improvements, but the opposite. Moreover, many of the crucial questions to which higher education has been waiting for answers remain to be decreed by ministerial order, with the result that past uncertainty will be prolonged. Those of Mr Baker's civil servants who have laboured through the summer on this turgid document should in fairness have called it something else.

The educational element in the bill is the proposal that there should be a national curriculum for the years of compulsory schooling, from 5 to 16. That makes just as much sense now as it did a decade ago, when a previous prime minister, Mr James (now Lord) Callaghan, and his then Secretary of State for Education and Science, Mrs Shirley Williams, organized what they called a "great debate" on just this issue, but failed to make their arguments stick in the face of the assertion by teachers' interests (and the local education authorities which, for the time being, run British schools) that a uniform curriculum would undermine their independence. The trouble has been that the movement towards comprehensive schooling that began in Britain in the late 1950s was too frequently taken by schools as a licence to let students follow wayward inclinations, not to learn mathematics for example. There will be broad support for the government's plan to put this state of affairs to rights, especially now that Mr Baker allows that there should be plenty of time within the school week for Latin and other sadly neglected studies. This is the bill's big plus.

Schools

On schools, there is also merit in the administrative proposal that head teachers should be free to spend their budgets as they see fit, free from detailed control of the local authorities that may continue to provide the funds. The risk is that school heads, usually at present appointed on academic grounds, may prove to be bad managers, while the boards of governors (among whom students' parents are to be strongly represented), now to be given charge of policy and appointments, may prove excessively concerned with short-term matters. To begin with, parents may be enthusiasts for change and improvement, but in the long term even schools are institutions requiring organic growth.

But these are not the issues that will keep Mr Baker and his men battling with the House of Commons in the months ahead. What will keep them from their beds are the interlocked proposals that students should be subjected to attainment tests at the ages of 7, 11, 14 and 16, that individual schools may opt out of local authority control and finance on a ballot of the students' parents for the time being, and that parents should have a right to choose the schools their children attend, subject to there being room for them. The first proposal, by itself a sensible means of monitoring the effectiveness of the national curri-

culum, will also be used by schools esteemed by parents to select the students who attend them, making British school education selective again. The provision for parental choice, outwardly democracy in action, will license racial, religious and class distinctions. The plan to give schools the right to be maintained by central rather than local government will be an administrative can of worms. Is it too late to persuade an over-zealous government that its national curriculum, and its proposals for more decisive management, should be given a fair chance in the system of largely comprehensive public education that has now emerged in Britain before the other divisive changes are foisted on British schools?

Omissions

The bill is even weaker in its omissions, especially on the interface between secondary and higher education. The tradition of requiring students in their last two years at school to rehearse the studies they will follow later on is the most dubious part of the British system. Administratively, there are significant changes already under way; the narrow school-leaving curriculum is being broadened (although it remains to be seen how universities will in practice respond). The Baker bill might usefully have attempted to define the function of this layer of the system, giving it an educational function not merely for intending university students but for the generality. Sadly, the government has ducked this issue, as it has also ducked the issue of access to higher education as such, what matters most to institutions of higher education, not to mention the young people most immediately affected by this bill. From time to time, no doubt, these matters will be decided by administrative decree.

On higher education, the bill is predictable but no more creditable on that account. The government would largely remove many of Britain's 26 polytechnics from the control of the local education authorities now responsible, making them virtually autonomous (which is good). But many of the more important questions remain to be decided by the Secretary of State for Education and Science, Mr Baker for the present. There are, indeed, to be a Polytechnics and Colleges Funding Council and a Universities Funding Council (no apostrophes, according to the draft bill) to channel money from the government to institutions whose remits are virtually identical — to provide higher and further (mostly vocational) education, to carry out research (and publish the results) and to do what else "may be prescribed" (by Mr Baker). But nothing is said in the bill about the threat (in April's white paper) that financial support will be provided only by means of educational contracts between the new paymasters and their dependants, except that there are provisions for making sure that institutions receiving money when there are strings attached will be required to repay it if the prescribed conditions are not met. The Secretary of State acquires the power to appoint the governing bodies of the newly independent polytechnics (except when these are statutorily elected) and also the power to abolish them. Nothing is said about the amounts of money that will be available.

British academics have become accustomed to all this in the past few months, although they have not yet learned to live with



it (which will be harder). Mr Baker's concept of a funding council is that of a 15-member corporate body, presumably an appropriate mix of Britain's great and good (voluntary public servants), whose functions will differ from the existing University Grants Committee in having no continuing responsibility to advise the government on policy on higher education (which means less influence), will have the power to hire its own staff (which is a plus), will have to disburse money to universities and polytechnics "subject to such conditions" as Mr Baker and his successors may determine (why should not the bill say what the rules are?) but may be as beastly as it likes to its dependants ("subject to such terms and conditions as they may think fit"). Whether the government will be able to persuade trustworthy people to serve on such a public corporation is open to question. It has needlessly complicated its task of finding compliant servants by distinguishing between the "six and not more than nine" members "appearing ... to have experience of, and to have shown capacity in, higher education" and the remainder, who will be people with "experience of, and capacity in, industrial, commercial or financial matters". British academics may be forgiven for thinking that the present government has been seeking to abolish these distinctions.

The bill will also seem, to many academics, to be mean on academic tenure, but that is not the case. Mr Baker has elected to follow the suggestion of his predecessor, Sir Keith Joseph, that there should be a body of University Commissioners empowered to amend university statutes so as to abolish the right to academic tenure. The proposal is a direct infringement of the autonomy of universities, although not an infringement of the academic freedom of individual academics, for the bill straightforwardly declares that fair grounds for dismissal include the fact that a university has given up the activity for which the person was employed, or that it has decided to cut back on it. There is nothing in the bill to suggest that academics may be fired because, while still able teachers and researchers, they hold unfashionable or even subversive opinions. The universities now say they hope to see these provisions amended; they would be better advised to see that new contracts of employment conform with the provisions of the draft bill — and to keep their powder dry for more important issues.

The chief of these is that the bill lacks educational content. Nowhere is there a phrase, let alone a sentence, to suggest that the present British government is aware of how a once-considerable educational system has fallen, by neglect and as a consequence of harassment, into structural disrepair and moral despair. The government does not attempt, even in the most general language, to define the objectives of higher education. (Schools are meant to promote "spiritual, moral, cultural, mental and physical development" and to prepare young people for "the opportunities, responsibilities and experiences of adult life".) As well as ducking the question of access to higher education, the bill says nothing about the functions of the different kinds of institutions the government will be running by ministerial directive in the years ahead. The government's failure to promise to take advice on some of these difficult questions is presumably predicated on the assumption that it will retain political power for the rest of time, which may be unwise. The neglect of this opportunity to unify the two halves of British higher education (universities and, broadly speaking, the polytechnics) belies the aptness of the word "reform". The casual balkanization of British higher education (Scotland and Wales will be differently dealt with separately from England) is a mistake. And the bill is badly drafted; although the University Commissioners will be able to amend the statutes of all institutions that have taken the government shilling in the past three years, the institutions subject to the Universities Funding Council are undefined. Many people had been hoping for better from Mr Baker. Before this long parliamentary session is over, many of his then-sleepless political colleagues in the House of Commons will share that view. □

Figures for research

Britain's research community must think harder about its funds, and how it spends them.

WHEN the British research councils had £500 million a year to spend, Mrs Margaret Thatcher, the prime minister, was fond of saying that "£500 million is a lot of money". Now, no doubt, she says that £600 million is a lot of money. She is, of course, right. So why does the British research community perpetually consider itself to be impoverished? In this season of annual reports from the research councils (see *Nature* 330, 196; 1987 for three of them), the government's *Annual Review of Government Funded R & D* for 1987, out this week, gives a simple explanation: the research community feels impoverished because it is being impoverished — government research spending is declining.

The decline is only marginal, but the direction of the trend accounts for the sense of hurt that has abounded in Britain throughout this decade. In terms of constant prices (those of 1985–86), spending by non-military government departments fell from £1,007 million in 1984–85 to an estimated £910 million in this financial year, more than the increase of the research councils' spending during the same period of £41 million. Even military spending on research and development declined over the same interval, by coincidence no doubt by the same amount as research council spending grew, to £2,226 million.

On the same showing, there will be no end to the misery. Having grown a little, research council budgets are expected to decline in the two years immediately ahead, to £551 million (at 1985–86 prices) in 1989–90. During the same period, the Department of Trade and Industry will spend an extra £33 million, but this will be more than offset by a further decline of defence research spending of £150 million, when the total cost of military research will be less than half of all the British government spends on research and development for the first time in half a century. Even though some of these shifts of spending may be desirable in their own right, their effects can only be to sharpen the sense that people have that they work in a declining field of interest. So, in Britain, they do.

The joker in this pack is the credit the British government takes (with some measure of justice) for the research component of what it pays each year to keep the universities going — £690 million (at 1985–86 prices) in the current year. If £600 million is a lot of money, £600 million plus £690 million is twice as much — that is what people may be saying. The trouble, of course, is that the government's contribution to research spending through the budgets of the universities is notional only; the sums concerned never materialize as disposable cash at the disposal of grant-making committees, but consist chiefly of a proportion of the salaries of all academics, all of whom are notionally supposed to be engaged in research.

So why not turn some of that budgetary money into real cash? During the past few months, British researchers have been looking enviously at what the universities are notionally spending on research. They are right to do so, but they will succeed in their ambition only when they have put their own houses in order. The largest of the British research councils, the Science and Engineering Research Council, with a total budget of £337 million, was able to spend only £100 million on research grants to independent researchers; the rest of its income went on external research collaborations and in-house services devised in happier times to be of service to independent researchers. If, by the time the Medical Research Council has reported, total disposable income for 1986–87 exceeds £140 million, most people will be surprised. The moral, for the research councils and the academic researchers who serve them, is that they must find a way of trimming these huge fixed costs. Then they might have a chance of persuading the government that a transfer of funds from the universities to the research enterprise — or from all universities to some — would be both equitable and wise. □

Blood free from retrovirus contamination: next step

- US blood banks begin testing
- Present tests too unspecific

Washington

BLOOD banks in the United States are getting ready to begin testing all donated blood for the presence of antibodies to HTLV-1, a retrovirus thought to cause adult T-cell leukaemia (ATL). Although the prevalence of HTLV-1 infection is still quite low, the virus has begun to surface in certain isolated populations — in particular intravenous drug users — and blood bank officials are anxious to minimize the threat that it might become more widespread through the use of contaminated blood.

Although rare in the United States, HTLV-1 is not uncommon in Japan. More than 1 per cent of the country's 9 million blood donors were believed to be infected with the virus before screening began a year ago (see *Nature* 323, 384; 1986), and as many as 35 per cent of the population of Okinawa may be infected.

In the Western Hemisphere, Jamaica also has a relatively high frequency of HTLV-1 infection. Caribbean studies have suggested that HTLV-1 infection may be responsible for tropical spastic paraparesis, a nervous system disease resembling multiple sclerosis.

But US blood screening may not begin for another year, because no test kit has been approved by the Food and Drug Administration (FDA). Three companies are developing screening tests; DuPont, Cellular Products and Abbott Laboratories. All are enzyme-linked immunosorbent assays (ELISAs), similar to the test at present in use for HIV (human immunodeficiency virus), the virus causing AIDS (acquired immune deficiency syndrome).

Preliminary data presented by DuPont at the meeting of the American Association of Blood Banks earlier this month showed that 167 out of 173 blood samples from ATL patients tested positive by ELISA. Of 24,759 blood donors, 33 (0.13 per cent) had positive ELISAs, but only five were confirmed positive by the presence of viral core proteins using a Western blot test. DuPont found the number of positive ELISAs among plasma donors to be higher — 60 out of 13,155 (0.46 per cent) — with 31 of these confirmed by Western blot.

In Japan, HTLV-1 testing is done using a particle agglutination test developed by Fuji Rebio. Gerald Sandler, associate vice president of medical operations of the Red Cross, says the Japanese test, although

reliable, is not automated. As the Red Cross must perform 6 million screening tests each year, an automated test is deemed essential.

Sandler says the tests are not as specific or as sensitive as he would like. But he says that the FDA has agreed to put the approval process for the kits on a 'fast track' basis. Sandler also says a good confirmatory test must be developed. Although a Western blot test is adequate to confirm a positive ELISA when screening for HIV, it often reveals proteins from only one HTLV-1 gene. Red Cross prefers to see proteins from three genes, so for HTLV-1 a confirming test may have to incorporate more sensitive approaches, such as a radioimmunoprecipitation assay. Only with a reliable confirmatory test will the Red Cross feel able to inform positive donors of their serologic status, and counsel them about the possible consequences.

The Red Cross will shortly release the results of its own study of HTLV-1 prevalence in some 40,000 blood donors, but Sandler emphasizes that the decision to prepare for testing is not in response to a current threat of disease or infection, but rather an attempt to forestall the spread of HTLV-1.

Sandler says the incidence of HTLV-1 infection among tested donors is of the same magnitude as that for HIV. At present, 8 of 10,000 donors are HIV-positive by repeated ELISA screening. Of these, 6 are not confirmed by Western blot, one is confirmed and one falls into an indeterminate category. This latter group has caused speculation that there may be another agent in the blood supply with core proteins similar to HIV. The one HIV-positive donor in 10,000 compares with 4 positive donors in 10,000 before the screening process began.

Although the frequency of HTLV-1 infection in the general US population is still quite low, the virus has invaded certain inner-city drug populations. A study of black drug addicts in New Orleans indicated that nearly half were infected with HTLV-1, and more than one-third of another group of addicts in New Jersey tested positive for the virus.

HTLV-1 testing is expected to have the added benefit of also identifying people infected with HTLV-2, a similar retrovirus that has been associated with hairy-cell leukaemia and T-cell chronic lymphocytic leukaemia. **Joseph Palca**

Good week for Soviet visitors to Britain

London

ACADEMICIAN G.I. Marchuk, president of the Soviet Academy of Sciences, said at the end of a visit to Britain a fortnight ago that he had been delighted with his delegation's discovery of the willingness of British scientists to collaborate with their Soviet counterparts. The Soviet delegation agreed with the Royal Society on regular visits at two-year intervals, alternately to Moscow and London.

The delegation appear to have been especially pleased with its reception at the universities of Glasgow, Edinburgh, Cambridge and Oxford and at Imperial College, London, where Marchuk says there were many people eager to take part in joint ventures with Soviet scientists.

Formally, the Soviet and British sides (the latter including the British Academy as well as the Royal Society) agreed to increase by a half the budget now available for financing the exchange of individual scientists, and to "work towards" a further 50 per cent increase a year.

One significant feature of this agreement is that the 25 per cent of the individuals exchanged under the extended agreement will be people nominated by the "receiving side" provided that they are "agreeable". The Royal Society hopes that this will enable British conference organizers to win the attendance of those from the Soviet Union whom they invite.

There is also to be increased attention to joint symposia and projects in certain fields. The reference in the joint statement to a discussion on the "free circulation of scientists" is understood to be a reference to the plight of a named *refusenik*.

The statement also says that the two sides will "make contact" with *Nature* and the Soviet magazine *Priroda* (published by the Soviet academy) to encourage the "exchange of materials on a regular basis". This is a reference to discussions under way for some two months.

The Soviet delegation included Academicians Y.A. Buslaev, director of the Institute of Chemical Physics (Moscow), L.D. Faddeev, director of the Leningrad branch of the Institute of Mathematics, Y.V. Galeev, deputy director of the Institute of Radio Engineering and Electronics (Moscow), B.K. Vainshetin, director of the Institute of Crystallography (Moscow) and R.V. Petrov, director of the Ministry of Health's Institute of Immunology. Also present were the president of the Georgian Academy of Sciences, A.N. Tavkhelidze, and corresponding member of the Soviet Academy A.A. Makarov, director of the Institute of Energy Research (Moscow). □

Universities unhappy about education reform proposals

London

BRITAIN'S universities have reacted with disquiet to the government's Education Reform Bill (Bill 53, HMSO, £10.50), fearing that the independence of institutions and individuals is being threatened. The clauses in the bill directly affecting higher education signal greater public accountability, increased central government influence and closer ties with industry. The bill contains no reference to funding through a system of contracts, as previously proposed, and there is no direct provision to protect academic freedom upon the abolition of tenure in the universities. The polytechnic sector has broadly welcomed the bill's provisions to allow polytechnics to manage their own affairs, without the imposition of local authority bureaucracy, and the narrowing of the gulf between state support of the universities and polytechnics.

The main provisions of the bill differ little from the White Paper (policy document) published last April (see *Nature* 326, 529; 1987). Polytechnics and certain of the larger colleges of further education will be taken from the control of local authorities to become statutory corporations. Finance will come direct from central government, distributed through a new Polytechnics and Colleges Funding Council (PCFC). The bill sets out in some detail the required constitution of the newly formed corporations' board of governors. It is likely that once independent of the local authority, an individual institution will be able to become a company limited by guarantee.

The mechanism for distributing public funds between universities will also change. The University Grants Committee (UGC) will be replaced by a Universities Funding Council (UFC) comprising 15 members, at least six of whom will have an industrial or commercial background (of the present 17 members of the UGC, two are from industry). The UFC and PCFC will have almost identical remits. The UFC, unlike the UGC, will not give to the Secretary of State advice by right on the financial needs of the universities, something about which the Committee of Vice-chancellors and Principals (CVCP) is "very concerned" and will be seeking to amend. The Association of University Teachers (AUT) has been taken aback by the stark wording of the clauses enabling the Secretary of State to confer on the funding councils "such additional functions as he thinks fit", and empowering him to attach conditions to any grants he makes to the funding councils. The funding councils in turn will be empowered to attach conditions to any payment they

make. An institution's failure to comply with any conditions would require it to refund (with interest) any payment made. These clauses, says the AUT, will "undermine the universities' traditional independence".

The bill makes no reference to contract funding. The significance of this omission is not yet clear. The Department of Education and Science thought the term 'contract' would introduce excessive legal and bureaucratic complications. Nevertheless, Education Secretary Kenneth Baker said that he wished to see a "contractual relationship" established between the funding councils and the institutions, although the contracts "would not be enforceable in courts of law". Tenure will be abolished by amendments to university statutes and charters by government-appointed commissioners. The changes would mean that academic staff could be dismissed for redundancy ("whether or not in pursuance of a scheme established for the purpose") or "for good cause". The absence of a specific clause safeguarding academic freedom has been condemned by the AUT and CVCP, who will be pressing for an amendment.

Simon Hadlington

Successful launch for Ariane

Paris

ARIANESPACE, the Paris-based company operating Europe's commercial space rockets, successfully launched an Ariane 2 rocket during the night of Friday, 20 November. Flight 20 placed the West German TV-SAT 1 direct-broadcasting satellite into geostationary transfer orbit. The satellite, the first of its kind in Europe, will enable four television channels to be received by householders throughout West Germany, including West Berlin (and therefore, in principle, East Berlin), part of Austria and eastern Switzerland, using only a small dish antenna.

The launch, the second since Ariane-space resumed commercial operations after problems with Ariane's third-stage motor, made use of the recently built second Ariane launch complex at Kourou in French Guiana. The success of flight 20 will bring a further boost of confidence to a nervous European space industry, and is certain to be interpreted by member states of the European Space Agency as confirmation of the wisdom of their recent massive investment in a long-term space programme centred around the next generation of Ariane 5 rockets (see *Nature* 330, 195; 1987).

Peter Coles

Arab-Israeli cooperation goes public

Rehovot

A COOPERATIVE science project that has been kept secret for five years was made public last week: Israeli and Arab researchers are working together in the struggle against cancer.

The French Cancer Research Association has been responsible for the cooperation, sponsoring colloquia on cancer in the Mediterranean. "The political climate has hitherto ruled out publicity", says Professor Michael Feldman, a participant from the Weizmann Institute in Israel. "Now there is some improvement in that climate, and so my colleagues and I feel that we can afford to go public." Feldman and Professor Nejib Mourali of the Salah Azaiz Institute in Tunis organized this year's colloquium in Paris, together with Professor Gerard Milhaud of the Saint-Antoine Hospital in Paris and Mr Jacques Crozemarie, president of the French Cancer Research Association. Among those who attended the meeting were scientists and physicians from Israel, Tunisia, France, Egypt, Morocco, Kuwait and Lebanon.

The potential for collaboration between Arab and Israeli researchers was evident from a presentation by Mourali and other Tunisians about inflammatory breast cancer, which accounts for almost 40 per cent of breast cancer in North Africa. A method used in Israel for measuring TNF (tumour necrosis factor) could be useful to cancer researchers in Tunis.

Scientific collaborations between Arab and Israeli researchers are extremely difficult to conduct because of the political animosity they generate from extremists on both sides. Even with Egypt, a country that has diplomatic relations with Israel, scientific exchanges are rare, despite areas of common research interest. For example, Israeli researchers have been interested in studies of urinary bladder cancer, a disease that is common in Egypt and is unusual because it causes far fewer metastases than do other malignancies. For their part, Egyptians have been closely following Israeli attempts to produce a vaccine against bilharzia, a debilitating, snail-borne disease endemic to Egypt.

Feldman looks forward to the day when Arab and Israeli scientists will meet freely, not only at colloquia in France, but also in each other's laboratories. But that day will have to wait "until the politicians get their act together", he says. "If it depended on the scientists, peace would long since have come to the entire Middle East."

Nechemia Meyers

'Industry should have final say in fate of research centres'

London

THE British government's evident wish for businessmen to dictate the direction of publicly financed scientific research will extend to the proposed university research centres if the chief science adviser in the Cabinet Office, Mr John Fairclough, has his way. The establishment of interdisciplinary research centres was one of the central recommendations by the Advisory Board for the Research Councils (ABRC) in recent advice to the Secretary of State for Education and Science, Mr Kenneth Baker, on ways to make more efficient use of the science budget (see *Nature* 328, 280; 1987). Although the concept of the centres has broad approval within academic and government circles, sharp differences are emerging on the question of the centres' management and control.

Speaking at a conference of businessmen and academics last week, Fairclough made it clear that the influence of the private sector over the centres is likely to be considerable. Institutions wishing to

research councils to discuss the commercial exploitation of academic research. The conference enabled the government to reiterate its well-known thoughts on the matter, articulated on this occasion by the Education Secretary. The national scientific effort should derive from a "partnership" between industry and academic institutions, whose achievement will require a "cultural change" from both parties. ("In talking with young scientists I have met some who are working in areas of strategic research but who have never discussed it with an industrialist. Some have never even had a business lunch," an incredulous Baker told the audience.)

Not all industrialists favour the government's approach. Professor John Cadoogan, of British Petroleum, holds that the function of a university is primarily to "produce excellent people at all levels and

produce the knowledge base on which industry can build", not to become "nothing more than a contract organization".

But Mr Anthony Gray, of a technology transfer company, Cogent Ltd, would like to see academic institutions adopt an "entrepreneurial and pro-active approach . . . if the full benefits of the wealth creation process of technology transfer are to be grasped".

The conference also focused on the question of ownership of intellectual property rights. Whereas industrial liaison directors in the universities favour the retention of the rights by the institution, with the granting of non-exclusive licences to commercial exploiters, many industries, unsurprisingly, prefer to obtain the rights, with an agreement to return them to the institution if after a specified time the rights had not been exploited. The Patent Office is presently running a 'general awareness' campaign aimed at academics, and the Department of Education is to review the field next year.

Simon Hadlington



John Fairclough encourages business.

house a centre would bid for government funds with the principal criterion being "the strength and extent of the partnership with industry". Approximately 80 per cent of the research budget would go on 'strategic' science and 20 per cent on 'basic' science. There would be a 'sunset clause' agreed at the beginning of the centre's life which would require the centre's work to be reviewed after a specified period (probably between five and ten years), when a decision would be taken either to continue the work or to close the centre. "The principal judge in this review would be the industry partners." This view seems to be at odds with the perception of the ABRC, whose strategy advice says: "We believe the prime responsibility for considering the progress of research within the agreed framework of objectives should rest with the research managers closest to the work."

Fairclough was speaking at a conference entitled 'Wealth from Science', sponsored by the Patent Office and the

Confusion over forecasts of Japanese volcanic eruption

Tokyo

MOUNT Mihara, a volcano on Oshima island south of Tokyo, has blown its top again a year after a major eruption forced the temporary evacuation of the island (see *Nature* 324, 295; 1987). But, despite an array of sophisticated monitoring equipment, Japan's Volcanic Prediction Council is divided over the interpretation of the latest series of eruptions.

Oshima is the first in a chain of volcanic islands that stretches south for over 1,000 km along the edge of the Philippine plate. In mid-November last year, the volcano burst into a series of violent eruptions that led to the evacuation of the 10,000 inhabitants of the island.

At the time, the prediction council came under fire for its failure to forecast activity of the volcano. And in December last year, more than ¥1,000 million (\$8 million) was spent on new seismic equipment. The island now bristles with dozens of state-of-the-art seismometers and tiltmeters, earning it the nickname 'hedgehog'. But the volcano's behaviour is still proving difficult to predict. First signs of activity came 2 weeks ago as minor earthquakes shook the island, and the Meteorological Agency warned residents to watch for future reports. On Monday of last week, hours before the prediction council was due to hold a meeting to discuss the latest seismic activity, the volcano burst into life, spewing plumes of ash thousands of metres into the air.

At a hurriedly convened press conference, two members of the council said it

was difficult to determine whether the eruption was the beginning of a series or a single isolated phenomenon. But Professor Yoshiaki Ida, head of the Izu-Oshima division of the council, said he thought that eruptions would continue. The next day, when the volcano had calmed down, an agency official explained that the eruption had been caused by a buildup of gas under the rocks piled up in the crater, and that, although similar eruptions might occur, the chance of a large eruption was very small.

Early on Wednesday morning the volcano burst into another series of four violent eruptions, the crater collapsed about 100 m, and clouds of ash were again thrown thousands of metres into the air. In a 5-hour marathon session the same day, the council agreed that Wednesday's eruptions had been brought about by an increase in pressure at depth due to magma movements which caused the northwest side of the volcano to rise and the shore of the island to sink immediately before and during the eruptions. But the council is divided as to why the pressure at depth rose; some council members think lava from the old crater sank back into the magma chamber, while others say new magma came up from depth. At present, the council does not expect a major eruption with lava overflow, as happened last year, but in the long term such a possibility cannot be ruled out.

The islanders are taking no chances. On Saturday they held a practice drill for evacuation of the island. David Swinbanks

Bavarian AIDS case to to to highest West German court

Munich

THE next battle in the West German AIDS (acquired immune deficiency syndrome) controversy will be fought in West Germany's highest court, the Bundesverfassungsgericht, or federal constitutional court.

A 46-year-old United States citizen convicted by a Bavarian court of inflicting "grievous bodily harm" on three sexual partners by not informing them that he carried the AIDS virus is now appealing, giving the *Bundesverfassungsgericht* its chance to join the fray.

The controversy, which has raged since the federal election in January that returned a conservative/liberal coalition to power, pits Health Minister Rita Süßmuth (Christian Democrat) and others against her coalition partners in Bavaria, the Christian Social Union (see *Nature* 325, 650; 1987 and 327, 264; 1987).

A decision handed down by the Landgericht Nuremberg-Fürth on 16 November sentenced the defendant, who has been in custody since February, to two years in prison. The prosecution had asked for a sentence of three and a half years. The case is seen as a landmark in deciding the legal liability of a human immunodeficiency virus (HIV) carrier who does not inform sexual partners of his or her condition.

The court called upon the West German government to add AIDS to the list of notifiable diseases, which already includes syphilis, gonorrhoea and other sexually transmitted diseases. People who are diagnosed with these diseases and who refuse to be treated for them must be reported to health authorities by name. They can then be forced to undergo treatment.

Süßmuth and her allies in the Social Democrats and Greens, currently in the opposition, reject the call to make AIDS notifiable, as there is no known cure for the disease. A registration requirement would just drive HIV carriers underground, they argue. Bavarian state secretary Peter Gauweiler, who has led the Bavarian crusade against AIDS, supports the measure.

The defence in the Nuremberg case asked for an innocent verdict, on the grounds that the defendant's sexual partners knew the risks of having sexual relations in the age of AIDS. But the court ruled that HIV carriers have a responsibility not to pass on the virus. Even though the defendant used a condom during ejaculation in all three cases, some risk remained.

The court ruled that even this "residual risk" should not burden an unwitting

sexual partner. A HIV carrier should practise "safe sex [i.e. use a condom during the entire sex act] or no sex at all", announced the court. In none of the cases could it be proved that the partner had

been infected with the virus.

Wilfully spreading AIDS can already be punished under West German law with enforced "isolation", although no case has as yet gone that far. Bavaria is the only *Land* to have expressed an intention to apply this law to HIV carriers who behave in an "unreasonable" way, such as prostitutes who do not use condoms.

Steven Dickman

New mirror furnace takes first spin

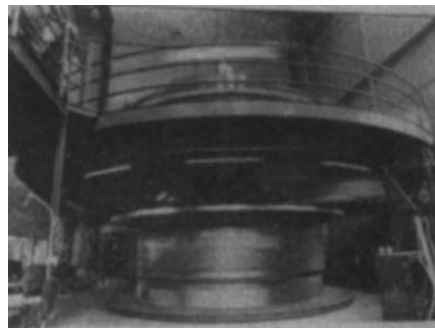
Tucson, Arizona

MIRROR builders at the University of Arizona Steward Observatory fired up their massive rotating furnace for its first mirror-casting test on 9 November. This first attempt will produce a 1.2-m parabolic mirror intended for a Smithsonian Institution telescope at Mount Hopkins, south of Tucson, but the new furnace will eventually be expanded to accommodate 6.5-m and 8-m mirrors.

Spin-casting mirrors — as the technique developed by the Arizona astronomer J. Roger P. Angel is called — promises to revolutionize mirror production. When the 5-m mirror in the Palomar Observatory's Hale telescope was made in 1948, it took a year to cool and was much heavier than spin-cast mirrors will be. The spin-cast mirrors will need far less extensive grinding and polishing and will cool in only about four weeks.

To start the process, 450 lb of glass was heated to 1,180 °C by 3,000 feet of electric

heating coils in the oven's walls, floor and lid. When the temperature reached 900 °C, the 23-ton steel turntable began rotating the furnace 8.5 times per minute for almost



Rotating furnace

15 hours to spread the glass over its mould and into a 1.2-m diameter parabolic shape.

This mirror is the third produced by spin-cast technology. If it is successful, the mirror laboratory plans to make two 3.5-m mirrors.

Elizabeth Pennisi

Call for government re-think on radiation dose limits in Britain

London

THE British government seems likely to amend its present radiation dose limits following advice* from the National Radiological Protection Board (NRPB) based on information from a preliminary reassessment of fatal cancer in the survivors of the atomic bombs at Hiroshima and Nagasaki.

In September, the International Commission on Radiological Protection (ICRP) concluded that the results of the Japanese study raised the risk estimate for the exposed population approximately twofold. The ICRP decided against immediately recommending a change in dose limits, and will not issue new advice until it has completed a review, likely to take at least two years. Pre-empting any advice from the ICRF, the NRPB is recommending an annual dose equivalent limit of 15 millisieverts for radiation workers and 0.5 mSv for members of the public (the present limits are 50 mSv and 1 mSv, respectively).

Re-evaluation of the Japanese data means that risk associated with continu-

ous exposure at the occupational dose limit of 50 mSv per year has increased from 1 in 2,000 per year to about 1 in 700 per year — a level of risk that "verges on the unacceptable", according to Roger Clarke, NRPB's director. About 2,000 people work near the dose limit — 1 per cent of workers whose exposure to radiation is monitored.

For members of the public, continued exposure at 1 mSv per year gives a risk of 1 in 100,000 per year on the old risk estimates and 3 in 100,000 per year on the new risk estimates — again verging on the unacceptable.

The board's criteria for 'acceptability' are based on the results of a Royal Society study group report on risk assessment, published in 1983. The Health and Safety Commission, one of the agencies with a regulatory responsibility, immediately announced its intention to re-examine radiation control measures in the light of the NRPB's advice. **Simon Hadlington**

*NRPB-GS9, *Interim Guidance on the Implications of Recent Revisions of Risk Estimates and the ICRP 1987 Compendium*. (HMSO, £3.00.)

Satellite-linked tsunami warning to avoid Pacific disasters

Washington & Tokyo

ALTHOUGH tsunami is a Japanese word, and the phenomenon, commonly known as a tidal wave, has killed more people and done more damage in Japan than anywhere else, all Pacific nations are at risk from its destructive power. Warning systems of varying sophistication exist in some, but many of the poorer or more remote areas, such as the Philippines and the west coast of South America, are largely unprotected.

Now a new system, developed by the US National Oceanic and Atmospheric Administration (NOAA), in cooperation with the Chilean authorities, is operating in Valparaiso, Chile, and may soon guard vulnerable sites around the Pacific rim for as little as \$20,000 per location.

Unlike the movie versions, real tsunamis are not walls of water several storeys high. Their height is modest, a metre or two at most, but they can be 100 miles long, and travel at 600 miles per

warning was not issued until 13 minutes after the original earthquake. Over one hundred people died.

The Japanese system also suffers from a dependence on complex technology. Where adequate maintenance cannot be guaranteed or where communications are fragile (especially after an earthquake), a simpler and more robust system is needed. On these criteria, the Foreign Disaster Assistance section of the US Agency for International Development (AID) approached NOAA, which already operates a Pacific warning system based in Hawaii. NOAA in turn began a collaboration with the Chileans to improve existing tsunami protection plans at Valparaiso.

The new system relies on a simple accelerometer, rather than a seismometer, set up to trigger when an earthquake above magnitude 7.0 on the Richter scale occurs. An alarm is sent to a geostationary NOAA satellite, which relays the signal to a ground station in Wallops Island, Virginia. There the emergency routine goes into action, and a general warning is issued via the satellite, back to the ground station.

In repeated tests carried out over the past year, a prototype system at Valparaiso has worked with 98 per cent reliability and with a response time of about one minute.

The system has been developed under the guidance of Dr Eddie Bernard of

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

A car stranded on the rocks after the Japan Sea tsunami of May 1983 in Aomori Prefecture, northwest Honshu, Japan.

NOAA, who says its virtues are simplicity, low cost and independence. A ground station consisting of an accelerometer, a small personal computer and the receiving and transmitting devices can be constructed from off-the-shelf components for about \$20,000, with running costs about one tenth of that amount per year.

Each ground station is self-powered, and is not connected except by satellite to any other equipment. What happens at each site when the warning is issued is entirely in the hands of local authorities, although NOAA and AID have helped in setting up evacuation plans.

Destructive tsunamis are rare, occurring about once a year throughout the Pacific region. It is a difficult matter, says Bernard, deciding at what level the system should trigger; not all big earthquakes cause tsunamis, and if false alarms were issued too often, people would become inured to them. The designers of NOAA's system have erred on the side of caution, hoping that the first years of operation will allow refinement of the ground station set-up. **David Lindley & David Swinbanks**

Kyodo News Service

IMAGE
UNAVAILABLE
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REASONS

A scene of devastation on the shore of Aomori Prefecture after the Japan Sea tsunami of 1983.

hour. On reaching the shore, the huge volume of water can flood harbours and overwhelm coastal villages in minutes, throwing fishing boats and even concrete breakwaters inland.

Tsunamis are caused by submarine earthquakes (occasionally volcanoes), and most of the destruction occurs within 200 miles of the original disturbance. Seismic p-waves travel at 400 miles per minute, which gives less than 20 minutes warning of an impending tsunami for those places most at risk.

The present Japanese warning system has advanced seismometers around the country, which feed their information to six regional centres for analysis and the issuance of tsunami warnings, graded according to the severity of the threat. A drawback is the time taken for warnings to be sent out: in May 1983 a 'great tsunami'

Spread of 'socialist jaundice' worsens in Uzbekistan

London

THE Uzbek Academy of Sciences and the Moscow Main Institute of Virology have launched a crash research programme into a new form of hepatitis reported to be of epidemic proportions in the republic.

According to Dr Sanasyr Shavakhavov, deputy minister of health and chief public health officer for Uzbekistan, the virus strikes for the most part people of working age, and has a particularly severe effect on pregnant women. The infection spreads at an "explosive" rate, he told a reporter from *Pravda Vostoka* last week. Clinical trials have, however, already begun in Tashkent of a new gamma globulin, developed in Moscow, which it is hoped will prove effective against the disease. Until recently, hepatitis — known colloquially in Eastern Europe as 'socialist jaundice' — was not openly mentioned in the Soviet media, as discussion of it was viewed as hostile criticism of the Soviet health service. The *glasnost* campaign has revealed, that in Moldavia, for example,

the hepatitis-B virus has been found in 13 per cent of the population, and in some regions as many as 23 per cent. In many cases, it is officially admitted, patients have been infected with the virus while in hospital for surgery, due to cross-infection from contaminated syringes.

The new Uzbek hepatitis (which may possibly be identical with the 'non-A non-B' form reported elsewhere) has triggered mass quarantine in which what Shavakhavov has described as "the most diverse ministries and departments in Uzbekistan" are involved. Travel beyond the boundaries of the republic is subject to strict public health control, and all vegetable and fruit produce sent out of the republic is monitored. All large gatherings, assemblies or meetings are discouraged, a decision which will prove a further impediment to the current attempts by the Crimean Tartars, resident in Uzbekistan, to organize a campaign for the right to return to their historic homeland. **Vera Rich**

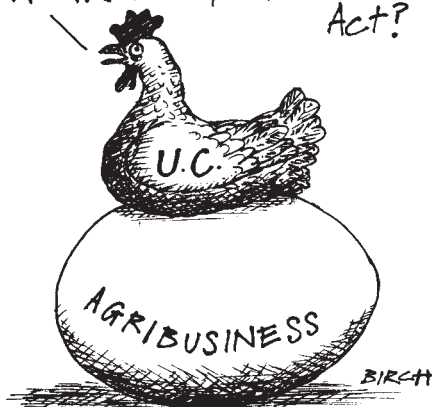
Encouragement in Japan for good forest management

Tokyo

THE International Tropical Timber Trade Organization (ITTO) based in Yokohama has made its first move towards encouraging improved forest management and sustainable use of tropical rainforests. Meanwhile, international environmental organizations have launched a campaign to halt destructive logging practices by Japan in Malaysia.

ITTO, a UN-sponsored council of 41 nations involved in the tropical timber trade, ended a five-day meeting on 20 November by approving a list of 12 projects on reforestation, sustainable management and market trends with a total

What's this if not a Hatch Act?



budget of \$2.8 million. Market trends for tropical timber trade in Europe, forest management in peninsular Malaysia, rehabilitation of previously logged forest in the Asia-Pacific region and sustainable management of an Amazon forest are among the projects approved.

The World Wildlife Fund provided \$10,000 for a \$100,000 project aimed at putting 100,000 hectares of Amazon forest in the state of Acre, Brazil, under sustainable management. But Dr Freezailah, executive director of ITTO, said there is still doubt among council members as to whether sustainable forest management is economically feasible on a large scale. Much will depend on major consumers of tropical timber, such as Japan.

Simultaneously with the ITTO meeting, Friends of the Earth International and the Japan Forest Action Network called on the Japanese timber trade to place a voluntary embargo on the importation of logs from Sarawak (Malaysia), to phase out importation of undressed logs, and to switch to importation of tropical hardwood that has been grown sustainably.

Japan is the world's leading importer of tropical wood. And 80 per cent of the imports come from Malaysia, nearly all of them in the form of unprocessed logs. Replanting and rehabilitation of defores-

ted land in Malaysia is minimal, and according to the World Bank and FAO (Tropical Forest Action Plan) the forests are in a "critical condition" due to over-exploitation and the forests of Sabah will be "commercially extinct" within less than five years.

Friends of the Earth intend to increase public awareness of the problem in Japan through, for example, signature-collecting campaigns. And the organization hopes to bring an end to wasteful practices, such as the use of tropical timber in

disposable plywood panels for concrete moulding in Japan's huge construction industry.

Koy Thomson of Friends of the Earth is confident that "when the Japanese consumers learn of the quite inexcusable and appalling environmental and social destruction caused by their timber trade, they will follow the example set by UK consumers who are refusing to buy destructively produced tropical timber in order to encourage the market in sustainable products". But the issue is not drawing media attention in Japan, and probably will not do so unless foreign governments, in particular the US government, begin to exert pressure on Japan, as in the case of whaling.

David Swinbanks

Too much agribusiness on California campus?

San Francisco

IN a decision that could have a wide impact on US agricultural research, a California judge has ordered the University of California (UC) to tailor its agricultural research programmes to benefit small farmers.

The judge ruled that the Hatch Act, an 1887 law that provides research funds for the land-grant universities, requires that the research it supports must benefit family farmers. Land-grant universities received gifts of federal property from the government as a part of their endowment. The judge's interpretation of the Hatch Act could fuel challenges to agricultural research in other states.

UC plans to appeal against the ruling, and argues that such limitation on the direction of research violates academic freedom. Hatch Act funds constitute less than 4 per cent of UC's annual \$116.5 million budget for agricultural research, but the money is spread over nearly 40 per cent of the research projects.

The lawsuit, filed in 1979 by a group of

small family farmers and farm workers, grew out of dismay over the mechanized tomato picker, developed in the early 1960s by agricultural engineers at UC. More than 30,000 farm workers lost their jobs as a result of the introduction of the tomato harvester in California in the early 1970s, says Bill Hoerger, attorney for the plaintiffs, and the number of tomato farmers in the state dropped from 4,000 to 490, although acreage planted in tomatoes doubled.

Concerned that UC was conducting agricultural research that primarily benefited larger farming corporations, the group filed a lawsuit claiming a variety of inappropriate relationships between UC employees and agribusiness companies. All charges but the Hatch Act violation were eventually dropped.

The plaintiffs were opposed to mechanization research, as well as to studies, among others, designed to produce uniformly ripening crops, a first step, they argued, toward the development of machines to harvest those crops. UC representatives argue that uniformly ripening crops would be beneficial and work-saving for all farmers, not just the giants.

UC attorney Christine Helwick says the judge did not decide that mechanization research hurts family farms, but merely ordered the university to evaluate the effect of its research on those farms. Helwick says UC research projects do keep the small farmer in mind, and adds that the California Grange, an organization of small farmers, entered the suit on the side of the university.

The university's opponents feel, however, that if UC properly evaluates the social and economic effects of its projects, it will be compelled to shift its research emphasis towards projects designed specifically to help family farmers.

Marcia Barinaga

Serron sacked

Paris

THE sacking last week of Dr Bernard Serron, head of the French National health education committee (CFES), only a year after his appointment by Health Minister Michèle Barzach, has been connected by some French newspapers with the 'disappearance' of millions of information leaflets about AIDS (acquired immune deficiency syndrome) due to have been distributed throughout France. A confidential report presented to Barzach by IGAS (the general inspectorate of social affairs) is thought to contain several examples of mismanagement at CFES since Serron was appointed.

Peter Coles

Bleaching of Caribbean corals a cause for concern

Washington

AN unusual event is occurring in the Caribbean. Reef corals from Colombia to Bermuda are losing their symbiotic algae, exposing the pale underlying coral skeletons, and possibly leading to widespread coral death. Although similar events have been recorded in the past, marine biologists from the region visited a snowy Capitol Hill last week to persuade Congress of the importance of tracking this episode and trying to determine its cause.

Reef coral play an important role in the marine ecosystem. Not only do they protect shorelines by forming a natural breakwater, but they are also an important nutrient source for other marine life, producing between 300 and 5,000 grams of carbon per square metre per year. The first indications that corals were losing their algae — a process known as bleaching — came from observations in mid-July at the Islas del Rosario off the coast of Colombia. When stressed, corals will lose the nitrogen-fixing zooxanthellae that normally attach to their skeletons. Stress can come from high water temperatures, increased suspended sediment or nutrients, and hypersalinity. Pathogens may also be causes for the bleaching, or possibly some interactive phenomenon that renders the zooxanthellae more sensitive to ultraviolet radiation.

In written testimony presented to the Senate appropriations subcommittee

looking into the Caribbean event, Judith Lang, curator of invertebrate zoology at the Texas Memorial Museum of the University of Texas at Austin, claimed that the widespread distribution of the event "suggests that some large-scale physical factor(s) is involved". John Ogden, director of West Indies Laboratory of Fairleigh Dickinson University in St Croix, agrees that a physical explanation seems most likely. Ogden says the current event underscores the need for better communi-



Deathly pale — bleached coral should be studied.

cation between marine biologists and oceanographers: the explanation may already exist in oceanic and atmospheric physical data, but oceanographers have not responded quickly.

Ernest Williams of the University of Puerto Rico says the pattern of the bleaching suggests something other than a physical factor. Although water temperatures are cooling to a temperature more favourable for coral growth, Williams says the bleaching is continuing. He believes there may be some latent disease that is being stimulated by physical conditions. Williams has the most complete records of the current event, and has sent out some 500 letters asking different sites about their experiences.

Coral bleaching has occurred in the past, as have widespread mortalities among other marine animals. Three years ago, between 95 and 99 per cent of the sea urchin *Diadema antillarum* were killed in the Caribbean by unknown causes, although a pathogen is suspected as the deaths were extremely species-specific. 'White band disease' has killed 90 per cent of the elkhorn coral at Buck Island Reef National Monument in St Croix.

Senator Lowell Weicker (Republican, Connecticut), an enthusiastic supporter of the National Oceanic and Atmospheric Administration Undersea Research Program (NURP), made it clear at the hearings that he expects NURP to take steps to do something about the bleaching. A meeting is planned for December among Caribbean marine laboratory directors. Ogden is optimistic that the next phase will be to watch the coral recover.

Joseph Palca

CNRS gold awards

Paris

THE Centre National de la Recherche Scientifique (CNRS) has announced this year's gold medal (Prix d'Or) laureates. The award has been given to historian of science Georges Canguilhem and mathematician Jean-Pierre Serre.

Canguilhem, aged 83, is emeritus professor at the Sorbonne. He taught at Toulouse in the 1940s, and also studied medicine, submitting a thesis on "the normal and the pathological". For 20 years he was professor of the history of science at the Sorbonne. He received the Resistance medal for wartime service, the Légion d'Honneur and the Médaille Georges Sarton.

Serre, 61, was a member of the famous Bourbaki group and his contributions to mathematics range from topology to geometry. His decorations include the distinguished Fields medal, and he is also an Officer of the Légion d'Honneur. P.C.

New blood for museum

Paris

AFTER a 2-month delay, the French cabinet has nominated a new director for the Science and Industry Museum, at La Villette in Paris. He is Christian Marbach, currently president and director general of ANVAR (Agence Nationale pour la Valorisation de la Recherche), the French governmental agency set up to promote technological research.

'La Villette', as the museum is known, has been criticized by Research and Technology Minister Jacques Valade for costing too much since it opened in 1986 (see *Nature* 329, 96; 1987).

Marbach, who gained a reputation at ANVAR for sound but innovative management, is no stranger to this government's cutbacks in public spending: ANVAR's grant was halved when the government took office last year, but has crept up again with a change in policy towards technology research. Grants to La Villette were reduced by 11 per cent in last September's 1988 budget proposals. P.C.

AIDS report update

Washington

PROMPTED both by the success and the failure of its first effort, the US Institute of Medicine (IOM) has begun work on a second edition of its report *Confronting AIDS: Directions for Public Health, Health Care, and Research*. When released last year, the report generated massive press coverage, and its conclusions were widely embraced, but not put into practice by federal government.

A small panel chaired by Theodore Cooper of Upjohn will assemble the report, aided by some 70 correspondents writing about different aspects of the AIDS problem. The updated edition of the report is due early next summer. J.P.

New AFRC secretary



London

THE Agricultural and Food Research Council (AFRC) has appointed Professor William D.P. Stewart as secretary to the council from 1 January 1988. He succeeds the late Professor John Jinks who died in post in June 1987. Stewart is a plant and microbial physiologist interested in nitrogen fixation and cycling and has many links with AFRC; he is a past member of the AFRC Plants and Soils Research Grants Board and is Royal Society Assessor to the Council.

Sarah Hargreaves

Malaise of British science

SIR—The polemic of Theocharis and Psimopoulos against recent philosophers of science (*Nature* 329, 595–598; 1987) deserves to be widely read, and as widely quoted. Yet I urge that the way forward will not be to denounce the contributions of these philosophers, but to see them in perspective.

I teach a (very short) course in philosophy of science to honours science students in their final year. I feature Popper, Kuhn and Lakatos, and I use Chalmers' book: of the devils denounced by Theocharis and Psimopoulos, only Feyerabend seems to me too deliberately destructive to be worth attention. The others are seriously striving to identify aspects of the scientific process. If their accounts are unbalanced, it is up to us to balance, not dismiss them.

Popper began it all by his concern to distinguish good science from bad. He identified Einstein as good and Adler as bad by characterizing Einstein's predictions as *falsifiable* but not as false. Falsifiable predictions were simply firm, clear predictions; predictions which explicitly required that the contrary did not occur. If it had occurred, they would have been falsified and (Popper judged) Einstein, unlike Adler, would have accepted that he was wrong and not tried to talk his way out of the fact. "Falsifiable" did not mean "seeking to be falsified", it just meant "strong enough to stand up and be counted on one side, not the other". I share the view that the next stage of Popper's thought sees him following up certain ideas to unbalanced and therefore somewhat antirational conclusions, but this first, key perception is firmly on the side of objectivity and truth. This is the one bit of Popper that I teach.

The theory-ladenness of observation must be recognized — but I teach it from Hanson, rather than Popper. It is a feature of the human mind to which we must face up: you will "see" (notice as significant) not "what you are looking for" (that, of course, is prejudice) but "*the kind of thing you are looking for*" (its presence or its absence). Not to make our young people aware of this would be to send them out into the laboratories as more naive scientists, not as more objective ones. But no hard-nosed industrialist can object to this: if he enquires into our procedures, he should be keen to know not only what questions we propose to ask but what kinds of observation we shall accept as answering them.

Kuhn is not wrong either. Every now and then great theories fall, and are replaced. More minor ones fall daily. Moreover, ideas sometimes return, after periods in the wilderness. These processes are not irrational, as Kuhn, growing tired,

concluded, but they occur — and it is up to us to work out their rationality. Lakatos, with his "protective belts of auxiliary hypotheses" and his "positive versus negative heuristic" makes, it seems to me, a constructive first attempt. But there is much more work to do, if a positive account of theory-change, which satisfies both the observations of Kuhn and the aspirations of Theocharis and Psimopoulos, is to be constructed.

Meanwhile, however, it will be only our own bad salesmanship that will let the lack of this complete account lose us money. Every industrialist I know accepts that science, being merely human, does not provide certain knowledge of the fundamental structure of the world (although of course he assumes, quite rightly, that it is continually striving towards this). He is, however, a tacit disciple of a much earlier philosopher of science — Bacon. What the industrialist wants is "not knowledge of nature, but power over nature". That power, science provides; when we do good science we shall reach right conclusions about matters of fact even though it is always possible (as the recent philosophers have been pointing out) that we are being right for the wrong reasons).

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SIR—Philosophers have always been accused of corrupting youth and subverting the state. The fashion began in Athens when Socrates was tried and executed on those charges. The anxious, the ill-informed and the unimaginative among his contemporaries were unable to tolerate a man whose wisdom lay in knowing how little he knew and whose method was to pose uncomfortable questions about the status of 'what everyone knew'. The Commentary by Theocharis and Psimopoulos (*Nature* 329, 595–598; 1987), makes it clear that times have not changed.

The real tragedy of their analysis lies in how little good it will do for the cause of science. They argue that science is being starved of necessary funds and they blame philosophers for that state of affairs. Philosophers, they say, have not been enthusiastic enough about the ability of science to yield truth. Is there anything in these charges? Certainly, philosophers have often sought to analyse the nature of science rather than to celebrate some abstraction called 'the scientific method'. That is to say, philosophers have given free rein to their intellectual curiosity and sought disinterested knowledge. In so doing, they have merely behaved like scientists.

But Theocharis and Psimopoulos produce no evidence that anything philosophers have said — whether positive or negative — has ever had the slightest effect on government finance. The nearest they get to an argument is to commit the fallacy of concluding 'after that; therefore because of that'.

There is, of course, every reason for serious concern at the state of government support for scientific research. Philosophers, sociologists and historians of science share scientists' concern, and it is natural that they should do so. Anxieties about the crisis in science should be addressed directly to those who hold the purse-strings. They should not be deflected into attacks on academic colleagues whose disciplines are under similar pressure. It is both a sign and a cause of weakness not to recognize one's true enemy.

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SIR—Theocharis and Psimopoulos (*Nature* 329, 595; 1987) have described what they consider to be the fundamental factor in the decline of support for science, namely the failure to establish its epistemological basis. This rather theoretical assault comes, however, mainly from academics who have never practised science and cannot see its cultural value. It is the latter aspect, involving the sociology of science, to which we should pay attention.

A striking feature of the postwar scene is the steady decline in the calibre of scientists called upon to advise governments. The present adviser to the US president is a former deputy director of the National Aeronautics and Space Administration, a post to which he graduated from 'weapons expert', and whose role in the shuttle disaster raised numerous questions (at the time of the launch he was advising the president on public relations). Even more heavily underlining the divorce between knowledge and power is the 'Star Wars' proposal to militarize space, shown to be nonsense and unanimously opposed by practising scientists. When one puts this beside the the very menacing problems of global ecology, one might agree with Spengler in pronouncing the decadence of the West.

Ultimately we have to appeal to the good sense of the man in the street. There are good reasons for doing science: not only our appreciation of nature but our survival on this planet depends on it. If social and political institutions do not recognize this we must change them.

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Tectonics in almost real time

Very-Long-Baseline Interferometry has been used with remarkable precision to map the movement of the coastal strip of California. Has the time now come when other regions should follow suit?

FANTASY has of course no place in science, but speculation may have a part to play. This is the justification for what follows, which is an account of how the movement of California west (or seawards) of the San Andreas Fault has been followed virtually instantaneously (over the few years since the technique became possible) with the help of mobile radiotelescopes operating in conjunction with a few fixed instruments in the same region. Those looking for a fuller account of what has been done should consult T.A. Clark *et al.*, *J. Geophys. Res.* **92**, 12,741;1987.

There is naturally nothing new about the notion that radio interferometry might have an important part to play in cataloguing the relative motions of the continents. At its simplest, the technique is simply that of old-fashioned range-finding: put your eyes further apart by viewing a distant object through reflecting prisms at the end of a horizontal beam in front of you, and you will be able to measure distances more accurately than in unassisted binocular vision.

But if the object is infinitely distant, so that the greater separation of the binocular points of view does not assist the measurement of distance, you have a sporting chance of estimating the size of the distant object if you can collect phase as well as intensity information. And the better the precision, the more detailed the contour map of the emission intensity from the distant object that may be constructed. That, of course, is how maps of distant radiogalaxies are made.

So, turning the problem around, it is easy to see how the simultaneous observation of a distant fixed object by two radiotelescopes on the surface of the Earth can be used to estimate the distance between them. This has been an important objective of Very-Long-Baseline Interferometry (capitalized that way because the mnemonic is VLBI) for at least the past 15 years. There is a certain amount of breath being held by those who wish to know whether the Atlantic is really broadening by between 5 and 10 cm a year. The usual assumption has been that it would be necessary to use large and therefore fixed radiotelescopes to wring accurate geodetic information from radio-interferometric measurements, and that the best baselines would be very long, perhaps even interplanetary in scale. Clark *et al.* have a simpler tale to tell.

Everything hangs on the properties of

what is called the Mark III geodetic VLBI system, devised by the US National Geodetic Survey in 1977. The system is not so much a technology as a method of management. Two independent radiotelescopes with receivers operating at two widely separate frequencies (2.2 GHz and 8.4 GHz) observe not just one distant radio source for just a few minutes, but several sources over several hours.

Naturally, the geodetic people cannot expect such dedication of time from the managers of radiotelescopes whose objectives are broader than the mere measurement of the Earth, which is why the National Geodetic Survey established a number of radiotelescopes in the United States with Mark III specifications that could be used for substantial periods of time on geodetic work. Clark *et al.* have the good luck to report on the usefulness of an extension of this network — a system using two mobile radiotelescopes which have been trucked from one side of the San Andreas Fault to the other during the past seven years.

The results of the survey of the movement of the western sliver of California are quite remarkable. For precision, the mobile telescopes have worked in conjunction with fixed telescopes scattered throughout the western United States. The mobile dishes are merely 3 m and 5 m in diameter, and can apparently be relocated to new sites at distances of several hundreds of kilometres at intervals of two days. Each convoy consists of two vans, one for the dish and one for the electronics. In the study of California west of the San Andreas Fault, the strategy has been to reoccupy the same observing site at regular intervals.

Unsurprisingly, it turns out that the coastal strip of California is indeed moving north-west relative to the continental United States at a lateral rate of 5.0 cm a year (± 0.1 cm a year). It is more remarkable that the inferred directions of movement of different parts of the western strip of California appear to be parallel to the most pronounced faults in the immediate neighbourhood, whether the San Andreas Fault or some other. No doubt it is only a matter of time before the operators of the mobile radiotelescopes are able to compete with the laser ground-ranging people in the accuracy with which they can tell which parts of the San Andreas Fault move smoothly, and which are locked together as a potential earth-

quake source. It is particularly interesting that the inferred movements of the coastal strip relative to those of the fixed inland observatories are large enough to throw light on the changes taking place in the Basin and Range Province, the wasteland between the Rockies and the Sierras. Using a fixed point in the Mojave Desert (not nearly half-way between California and Arizona) as a reference point, Clark *et al.* conclude that, if coastal California is moving north-west at roughly 4 cm a year, Flagstaff (in Arizona) is moving in the opposite direction at roughly a third the speed. But if Arizona is the reference-point, the total movement of the coastal strip is apparently sufficient to attribute all the relative motion of the Pacific and the North American tectonic plates to slip along the San Andreas Fault and its congeners, without the need to invoke slip along a parallel system of offshore faults.

The relatively small errors of these measurements are a consequence of two independent considerations — the amount of time for which the mobile stations have been dedicated to geodetic observation (and thus the amount of data collected per unit time) and the total length of time over which data have been accumulated (four years seems typical). There can hardly be a more striking illustration of how, in the exploitation of a new technique, management may be as important as technology.

Two questions immediately arise, of which the chief is whether the errors of these measurements with mobile dishes, small though they appear to be, can be further reduced. Obviously that must be possible. Provided that a mobile radiotelescope can see a specified distant radio source, what matters is chiefly that it should be able to record the time variation accurately. Sensitivity may be less important than the accuracy of timing.

So should not systems such as that described by Clark *et al.* find some kind of application in other tectonic areas? There are few places where the rate of local movement is as great as 5 cm a year, but it is not unreasonable to hope that movements of the order of 1 cm a year will soon be measurable over intervals of a few years. The great fault systems of Central and Eastern Asia should be readily measurable by these means. While disaster prevention is as much the rage as now, is that not something to plan for?

John Maddox

Microfilament dynamics

Few answers but many questions

Harriet Harris

In many cell types, physiological activation stimulates the assembly of actin, the main protein of microfilaments. Because microfilaments are important contractile and skeletal components of cells, such a change is causally linked to a change in the cell's motile behaviour. Actin is almost completely polymerized (filamentous) under physiological ionic conditions, so the regulation of its assembly must be mediated by the action of specific actin-binding proteins, which in turn may be regulated by intracellular second messengers such as calcium, the levels of which are modulated by activator-receptor interactions at the cell surface. Several groups¹⁻³ are attempting to elucidate the sequence of these events in cells after activation.

One strong candidate for a regulator of microfilament assembly is gelsolin⁴, a protein that severs filaments in the presence of calcium (thus solvating actin gels, hence its name) and 'caps' the free barbed filament ends so produced (see figure). Barbed ends, so-called from their appearance on actin filaments decorated with myosin subfragment-1, are the faster-growing ends of the filaments and equilibrate with a lower steady-state monomer concentration than the pointed ends. The combined effects of severing and capping thus accelerate and enhance filament disassembly. On the other hand, gelsolin also accelerates assembly, binding two actin monomers to form a nucleus. Which of these functions, disassembling or assembling, does gelsolin facilitate *in vivo*?

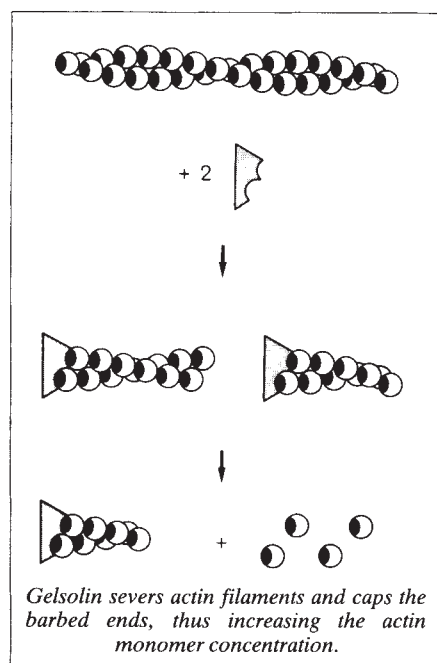
A further problem is the failure of gelsolin-actin interactions to reverse when Ca^{2+} is removed; how then can it participate in the cyclic regulation of microfilament dynamics in the cell? Here, a second messenger is required to complement the action of Ca^{2+} ; there is evidence that, *in vitro* at least, this function is fulfilled by the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (Ptd(4,5)Ins), which dissociates gelsolin both from small complexes⁵

and from capped filament ends⁶. This suggestion is particularly intriguing, as Ptd(4,5)Ins is the immediate precursor of the known second messengers inositol trisphosphate and diacylglycerol. In addition, it dissociates profilactin, the complex of profilin and actin that stabilizes a pool of actin monomers in cells⁷.

A synthesis of the combined *in vitro* properties of gelsolin, profilin, actin, Ptd(4,5)Ins and Ca^{2+} is utterly disconcerting. The naive prediction is that a cell stimulus, elevating Ca^{2+} and transiently depressing Ptd(4,5)Ins, would enhance filament severing, enhance the monomer pool by barbed-end capping, enhance formation of profilactin and, overall, promote actin disassembly. But what is observed is that, on the contrary, stimulated cells respond by assembling actin.

The way forward is more likely to come from finding out how proteins actually behave in cells than from predicting what they (apparently) ought to be doing, and the general approach adopted in the new papers¹⁻³ is to determine what happens in cells following stimulation. Carson *et al.*¹ show that neutrophil stimulation by a chemotactic peptide known to promote actin assembly increases the number of filament-nucleation sites in detergent lysates of cells. These new nuclei have the properties of free barbed ends of actin filaments, consistent with the loss of a capping protein such as gelsolin from a blocked, pre-existing filament. Barbed ends are more efficient nuclei than gelsolin-actin complexes, which allow growth only at the slower, pointed end.

Lind *et al.*² used a similar technique to determine the state of gelsolin and profilin in platelet extracts following thrombin treatment. Thrombin induces rapid increases in high-affinity complexes of gelsolin with actin and of profilin with actin. This behaviour is consistent with a transient decrease in Ptd(4,5)Ins and an increase in cytoplasmic Ca^{2+} , effected by the thrombin. Most significantly, the concentrations of these complexes fall later,



in parallel with the large-scale assembly of actin, into a detergent-insoluble cytoskeleton. An important feature of this phase is that free gelsolin is regenerated in the cytoplasm. It would be plausible to postulate that the recovery of Ptd(4,5)Ins to resting levels releases free gelsolin from newly formed oligomers, supplying an abundance of free barbed ends to nucleate filament formation from the bulk of monomers, simultaneously released from profilactin (see table).

The importance of these observations^{1,2} is that they offer a two-step model of a role for gelsolin in the initiation of microfilament assembly. One weakness is that the role of profilin remains unclear: it is assumed to contribute to the stabilization of a monomer pool within resting cells, but in a low-affinity complex not detected by the binding assay. The function of the high-affinity profilactin is entirely enigmatic. Furthermore, the model rests on observations on protein behaviour, and needs substantiating with evidence that the levels of the postulated second messengers exhibit appropriate changes coordinating precisely with the changes in protein interactions.

There is also a problem in invoking the function of Ptd(4,5)Ins as the agent dissociating gelsolin from actin filaments, namely that it resides in the plasma membrane, whereas free gelsolin and actin monomers are soluble proteins in the cytoplasm. Although it is reasonable to postulate that the relatively compact profilactin diffuses to the membrane to be dissociated, this mechanism becomes far more cumbersome for actin oligomers with gelsolin attached. It is also disturbing that Ptd(4,5)Ins is more effective in an unphysiological state (micelles) than when combined with membrane phospholipids⁶. Finally, the question of whether

A plausible model for events in thrombin-stimulated platelets

Resting cells		Free gelsolin Actin monomer pool (low-affinity profilactin)
Stimulus:		
Step 1 (15 s)	Ptd(4,5)Ins falls Ca^{2+} rises	High-affinity gelsolin-actin nucleates oligomers High-affinity profilactin
Step 2 (5 min)	Ptd(4,5)Ins rises	Profilactin dissociates Gelsolin released from oligomeric actin Free actin monomers assemble onto barbed ends of oligomers

gelsolin solvates gels *in vivo* when the cell returns from an activated to a passive state remains to be explored.

A third recent study³ adds another dimension to the problem of actin assembly, implying that cells contain functionally separable populations of actin, independently controlled by different second messengers. Certain neutrophil activators, including chemotactic peptides, induce a transient stimulus of actin assembly. This effect is inhibited by pertussis toxin, implying regulation by an as-yet unidentified G protein. On the other hand, phorbol 12-myristate 13-acetate, a potent activator of protein kinase C, or a drop in cytoplasmic pH, stimulates a slower but persistent and toxin-insensitive assembly. The effects of the alternative

types of stimuli are additive. This development will appear logical to cell biologists accustomed to observing individual cells perform different motile activities simultaneously, but it complicates the still considerable task of biochemists in pursuit of the complete answer to the question of what makes microfilament structure dynamic. □

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Circadian rhythms

Hamsters without jet-lag

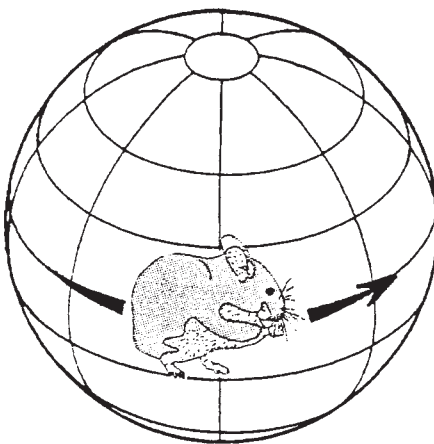
A.T. Winfree

ON page 372 of this issue¹, Mrosovsky and Salmon dramatize our ignorance of circadian physiology by an experiment in simulated 'jet-lag' using hamsters. Anyone who has travelled rapidly across time zones knows that internal physiological and behavioural rhythms do not adapt immediately to rescheduling of day and night. Experiments with circadian dynamics are done in laboratories with less waste of jet fuel by merely resetting an electrical clock that governs environmental lighting. Either way, if the change is no more than a couple of hours per day, then adjustment is probably mediated by the same process that maintains stability of entrainment during the usual unperturbed succession of dawns and dusks. If daylight today intruded slightly on normal pre-dawn stages of the cycle, and/or left in darkness stages that normally precede a timely dusk, then tomorrow the cycle will arrive at each stage a little earlier; or a little later if instead light had lingered longer than normal around dusk, and/or early morning stages normally illuminated were instead left in darkness.

What happens in the case of more abrupt displacements of the schedule is anybody's guess. Guesses have, of course, been ventured, many of them based on an unproven assumption implicit in the foregoing description. Observations are often interpreted as though photic or pharmaceutical stimuli caused an abrupt phase-shift of some cyclic sequence of events, and that the effect of a succession of repeated stimuli (for example, exposure to a rescheduled train of dawns and dusks) can be predicted by iterating the measured dependency of post-stimulus phase on pre-stimulus phase. If nature were as simple-minded as man,

science would progress more quickly than it does by theory alone; alas, this is seldom the case. Despite occasional success with well-known preparations, the process of re-entrainment generally remains more obscure for circadian clocks than it does for analogous idealized oscillators.

The observation¹ of Mrosovsky and Salmon provides an unanticipated hint. It consists, in essence, of a single datapoint on a landscape that will now have to



be filled out, and its meaning cannot be fully understood until that context is delineated. But the isolated fact so violates preconception that it merits immediate attention all by itself. Suppose you advance the timetable of dawns and dusks by eight hours, starting with a premature dusk. Under these conditions, hamsters usually require a week or so to adjust. However (and this is the new fact) they adjust almost immediately if you firmly set their feet on the path, by moving them to a clean, unfamiliar running wheel at the time of night when they will be

active in the future, although they were sleeping at that time in the past.

It would, of course, also be useful to know whether this is equally true for advances and delays of all sizes, how or whether the effect depends on the timing of the one forced activity, whether its magnitude diminishes as the duration of activity is diminished, whether behavioural preconditioning works only for subsequent entrainment by light stimuli, and whether the subsequent stimuli are even necessary. Until the answers are known, the result may seem a very small fact. But it does raise many questions.

Does changing the hamster to a different running-wheel for three hours only enhance re-entrainment, or does it enhance a specific re-entrainment job by causing a specific large phase-shift of the circadian clock? In a simultaneous paper², Mrosovsky addresses this problem using similar, though briefer, stimuli, and did find phase-shifts, but of much smaller magnitude. So does wheel-change followed by a photoperiod (neither of which alone inflicts much phase resetting) constitute a more potent resetting stimulus? The phase-shifts reported in this issue¹ appear to approach 8 hours, which for almost the first time³ would put a vertebrate species into the realm of circadian dynamics explored heretofore only in protists, fungi, plants and invertebrates; the realm of large phase-shifts in which phase alone is often not a sufficient description of the state of 'the clock' and in which such peculiar phenomena as phase singularities were first predicted, then copiously found⁴.

If wheel-change in unexpected darkness did cause a large sudden phase-shift, then we must find out what other behavioural experience can also wreak such dramatic effect on circadian processes. We might discover that inadvertent poor timing of those experiences is responsible for various circadian arrhythmias, such as insomnia. Could the effects of more-studied photic or pharmaceutical stimuli be mediated, not directly by molecular encounters with the clock mechanism, but indirectly through their effects on behaviour? Is the putative phase-shifting effect of behaviour actually a clue to the specific chemical role of some neurotransmitter in the clock mechanism?

But could the observed effect have nothing whatever to do with phase-shifting? Could the behavioural experience instead be resetting only the amplitude of circadian oscillation, perhaps so near to zero that the next familiar stimulus has immensely magnified impact on phase and, finding the clock delicately poised near equilibrium, reinitiates rhythmicity from the outset in standard phase relation to the new light/dark cycle? The activity rhythms of similar experiments² often show more conspicuous rearrangement of



100 years ago

ANOTHER contribution to the subject of photography in colours is published by Mr. Carey Lea in the November number of the *American Journal of Science*. Mr. Lea finds that silver chloride combines with small quantities of many other chlorides, besides its own sub-chloride, to form coloured salts, comparatively stable and remarkably less sensitive to light. Thus if silver nitrate be added to a solution of ferric chloride in presence of free hydrochloric acid, the precipitate obtained is buff-coloured, and the ferric chloride carried down by the silver chloride cannot be washed out even by hydrochloric acid. The most remarkable property of this silver-ferric chloride is that it is almost unacted upon by light.

From *Nature* 37, 88; 24 November 1887.

pattern and amplitude than of phase.

Or could the experience of wheel change have no direct impact on clock dynamics at all, but only heighten the animal's alertness to timing cues, perhaps specifically to visual cues, by which to re-schedule sleep and waking?

And what about human jet-lag? Generalization from the activity rhythm to diverse unobserved physiological rhythms, or from rodents to humans, seldom turns out well when specifics are involved⁵, but Mrosovsky and Salmon legitimately raise a general question for empirical resolution: could suitably chosen voluntary behavioural experience assist re-entrainment in humans as strikingly as in the hamster? Would getting angry or making love (if phase-shifting is involved, then perhaps necessarily at the right time in relation to the circadian clock's phase at first dawn or dusk in the new time zone) put our neurotransmitters and suprachiasmatic nucleus on the optimum trajectory? My own habit is to go running in bright sunlight at hours estimated to nudge my shifting clock's phase in the intended direction, and to avoid such exposure at hours when the effect would be opposite. Superstitious or not, it seems to work for me; but is it the sunlight, the running, the timing or the belief that is important? With so many aeroplanes continually crossing time zones, opportunities to find out are being missed every day. □

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Oceanic crust

Slow waves in young basalts

Peter Shearer

A UNIQUE machine that detonates explosive charges just above the sea floor helps to provide some of the best data to date about the seismic velocity structure at shallow depths in very young oceanic crust. The results, discussed in a recent paper by Purdy¹, indicate that the velocity of compressional waves (P waves) at the surface of young crust on the Mid-Atlantic Ridge is only 2.1 km s^{-1} . This is much closer to the velocity of the overlying ocean (1.5 km s^{-1}) than to that of sea-floor basalt samples (5.7 km s^{-1}), implying that the large-scale porosity of fresh basalts exposed at the sea floor is up to 30–50 per cent, much higher than previously thought.

Conventional seismic refraction experiments, in which the explosive sources are detonated near the sea surface, are poor for resolving seismic velocities within the top few hundred metres of the oceanic

Hole Oceanographic Institution have taken the latter approach, and have developed a deep-towed explosive source (DETES) capable of firing up to 48 individual explosive charges within 100 m of the sea floor (see figures)³. On command from the overlying ship, 2.3-kg charges are first released to hang 30 m below the device and then detonated, using an explosive that is reliable at high pressures. A hydrophone on the device records the explosions to ensure that the timing of the shot instant is known precisely. A further ocean-bottom hydrophone is anchored to the sea floor nearby. In a typical experiment, this ocean-bottom hydrophone is first released onto the sea floor by the DETES, which is then towed away slowly, firing a series of charges at increasing ranges from the receiver.

The first DETES experiment was conducted in 1985 above a small volcano within the inner floor of the Mid-Atlantic Ridge near latitude 22°N . The results indicate a P-wave velocity of 2.1 km s^{-1} at the surface of the crust and a near-surface velocity gradient of approximately 4 s^{-1} within the top 200 m of the crust. Low seismic velocities and steep velocity gradients near the surface of the oceanic crust have been observed before, but these new results are better constrained than those from earlier experiments which did not use ocean-bottom sources, and the velocity is much lower than that indicated by most previous work. In contrast to these results for new crust, additional DETES experiments which were conducted 14 km away on 7 million-year-old crust indicate a surface P-wave velocity of 4.1 km s^{-1} and a velocity gradient of less than 0.5 s^{-1} .

Low P-wave velocities in the shallow oceanic crust have generally been explained as an effect of large-scale pores and cracks within rock of higher velocity^{5–7}. The steep increase in velocity with depth in the top kilometre of the crust is thus related to the observed decrease in porosity in this layer⁸. In a similar way, the seismic velocity at the surface of the crust is thought to increase with age as a result of the slow cementing of cracks within the rock⁹. Purdy's results are consistent with this general model of the structure and evolution of the uppermost oceanic crust,

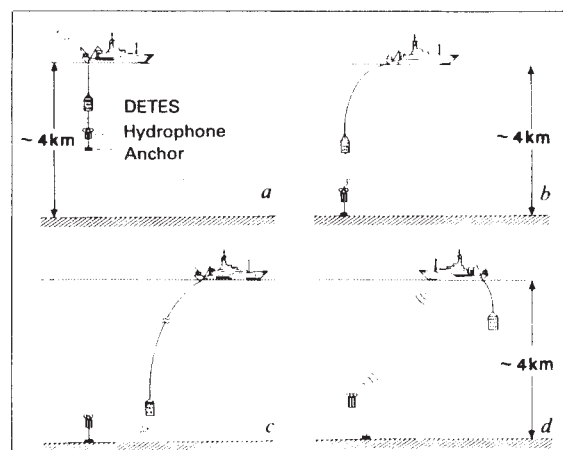


Fig. 1 Cartoon showing the operation of a DETES experiment. *a*, The deep-towed explosive source (DETES) and an ocean-bottom hydrophone are lowered to the sea floor. *b*, Close to the ocean floor, the hydrophone is released to free fall to the bottom. *c*, The vessel moves slowly away with the DETES firing charges at regular intervals, generating seismic waves which are recorded by the hydrophone. *d*, The DETES is hauled in and the hydrophone is released from its anchor to be recovered on the surface. (From ref. 1.)

crust. A more direct measurement is possible if both the source and receiver are located near the sea floor. Ocean bottom receivers (seismometers or hydrophones) have been used for years, but until recently seismic sources were nearly always located near the sea surface because of technical difficulties in using sources at great depth, such as timing accurately the instant of the explosion and the unreliability of most explosives at high pressures. Recent attempts to solve these problems include free-falling 'pipe bombs' with timers² and devices towed deep beneath ships that can sequentially ignite a series of charges^{3,4}.

Purdy and co-workers at the Woods



Fig. 2 The explosives platform of the DETES seismology apparatus used by Purdy and co-workers for high-resolution studies of young oceanic crust. (From ref. 1.)

but the observed velocity is so anomalously low that it implies the new crust is extremely porous, because laboratory analyses of basalt samples from the Mid-Atlantic Ridge consistently indicate velocities of $5.7\text{--}6.0\text{ km s}^{-1}$.

Theoretical relationships between seismic velocities and porosity depend heavily on the shape of the pores and cracks in the host rock^{5,6}. For a given velocity decrease, however, it is possible to determine the minimum porosity that is required, regardless of the shape of the cracks⁷. Assuming a host-rock velocity of 5.7 km s^{-1} and water-filled cracks, a minimum porosity of 22 per cent is required to reduce the velocities to the observed 2.1 km s^{-1} . But the predicted shear modulus for such a material is zero, which is incompatible with the observed shear waves from earthquakes recorded by ocean-bottom seismometers on the Mid-Atlantic Ridge¹⁰. The porosity must be higher than 22 per cent for the shear modulus not to go to zero, and Purdy speculates that it is as high as 30–50 per cent. No one has yet measured directly the porosity of new crust, but borehole measurements in 7 million-year-old crust indicate porosities of only 10–15 per cent. If younger crust is more porous, then there must be a process that reduces significantly near-surface porosity soon after the formation of oceanic crust. □

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RNA splicing

Helping RNA catalysis along

L.A. Grivell

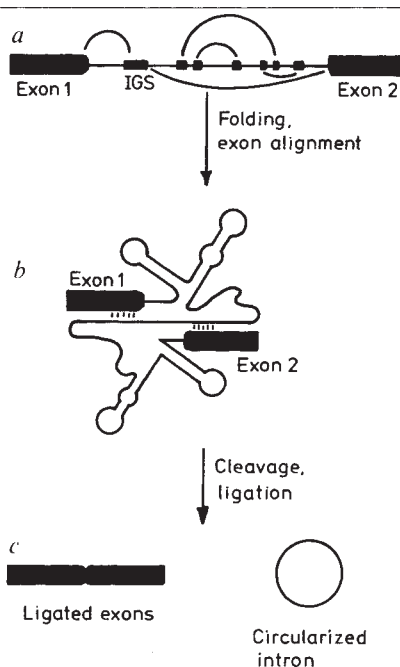
SINCE the pioneering work of Thomas Cech and his colleagues on the self-splicing reaction carried out by the precursor to the large ribosomal (r)RNA in the protozoan *Tetrahymena*, the principle of RNA catalysis has become a familiar one (see ref. 1 for a review). When incubated in a simple salt medium, in the presence of a guanosine nucleotide, the intron in this RNA can catalyse many reactions involving the breakage and re-joining of phosphodiester bonds in RNA with an accuracy and efficiency that was once considered to be the exclusive prerogative of proteins. Despite this versatility *in vitro*, however, there is good reason to believe that *in vivo*, the reactivity of this RNA and others like it is tempered by the action of proteins. Nowhere is this more clearly apparent than in the splicing of introns in fungal mitochondria, where a decade of genetic and biochemical analysis has uncovered requirements for many proteins, some of them intron-encoded, in intron excision (see ref. 2 for a review). The latest instalment in this fascinating story is described in a recent paper³ by Akins and Lambowitz.

The authors have isolated and sequenced a nuclear gene required for splicing of several introns in *Neurospora crassa* mitochondrial precursor RNAs, some of which have self-splicing activity *in vitro*. The encoded protein turns out to be the mitochondrial tyrosyl transfer (t)RNA synthetase. This protein, or a derivative of it, possesses both splicing

and amino-acylation activities.

The intron studied by Akins and Lambowitz³ lies in the gene encoding the large rRNA in *N. crassa* mitochondria. Its predicted secondary structure and conserved sequences within it label it as a group I intron, together with several others in the *Neurospora* mitochondrial genome. Although classification as such means that it in principle possesses all features thought to be necessary for self-splicing *in vitro* (see figure), excision *in vivo* and *in vitro* is dependent on proteins. Previous work from the same laboratory has identified temperature-sensitive nuclear mutations responsible for a deficiency in splicing at the non-permissive temperature of 37°C ; mitochondrial lysates prepared from one of these mutations, *cyt 18*, are deficient in a soluble activity that functions in splicing *in vitro* and this mutation has been characterized further⁴.

Initial gene cloning by complementation of the *cyt 18* mutant, followed by judicious sub-cloning, shows that the gene corresponds to a 531 amino-acid reading frame, interrupted by a short intron close to its amino terminus. The sequence resembles those of tyrosyl tRNA synthetases from both *Escherichia coli* and *Bacillus stearothermophilus*, with 28.4 and 25.3 per cent identical residues, respectively. Armed with this knowledge, Akins and Lambowitz³ checked tyrosyl tRNA synthetase activity in the *cyt 18* mutant and found it to be severely reduced in cells grown at 37°C . At all



Stages in the splicing of a group I intron. *a*, Black boxes, exons; solid line, intron; boxed areas within the intron, short, conserved, complementary sequences involved in intron folding. One of these, the internal guide sequence (IGS), is capable of base pairing with regions of both exons and is responsible for exon alignment before splicing. *b*, Intron folding results in exon alignment along the IGS (base pairs shown as short vertical lines) and creation of a catalytic centre, to which a guanosine nucleotide (not shown) can bind. Binding triggers a series of transesterification events which result in cleavage and ligation reactions (see ref. 1). *c*, Transesterification results not only in exon–exon ligation, but also in intron circularization. In this latter step, a small, 5' terminal segment of the intron (not shown) is split off. It is not yet known which stage of splicing is dependent on the product of the *cyt 18* gene.

temperatures assayed, splicing and aminoacylation activities paralleled each other, suggesting that the *cyt 18* gene product is involved in both processes.

One way in which a defect in a tRNA synthetase could interfere with splicing is indirectly, by way of a general block on mitochondrial protein synthesis and hence energy metabolism. This mechanism seems not to be the case in *cyt 18*. In a set of selected revertants to splicing proficiency, secondary mutations in the reading frame restore splicing activity, even though both aminoacylation and mitochondrial protein synthesis remain low. These results indicate that the link between the tRNA synthetase and splicing is a direct one: the synthetase is thus either a component of the splicing machinery, or is somehow involved in the regulation of its synthesis or stability. The results also show that splicing is not directly dependent on aminoacylation activity.

What is the role of the *cyt 18* product in splicing? There is as yet no clear answer to this question, although Akins and Lambowitz provide some intriguing clues. First, ribonucleoprotein particles isolated from *Neurospora* mitochondria are enriched in tyrosyl tRNA synthetase activity; partial purification of splicing activity from these separates tyrosyl tRNA synthetase activity into two main peaks, only one of which is associated with splicing. Thus activity may be attributable either to a specific quaternary form of the synthetase, or to a complex formed between it and other protein(s). It is to be hoped that further purification will resolve these alternatives.

Clues to a mechanism of action are also given by observations that point to the importance in splicing of the amino terminus of the protein. Most important of these is the finding that in one *cyt 18* mutant, splicing deficiency results from the change of the glycine at position 127 to glutamate. This residue corresponds to the conserved glycine at position 58 in both the *E. coli* and *Bacillus* enzymes, in which it forms part of the domain involved in adenylate formation (see ref. 5).

Akins and Lambowitz speculate that the *cyt 18* protein recognizes common tRNA-like structures in the introns whose excision it helps catalyse. So far, however, there is no indication that the protein makes direct contact with the intron RNA. Given that the functional part of the *cyt 18* protein is its amino-terminal adenylate-forming domain, rather than that involved in tRNA binding, other types of interaction may be more likely.

Surprising though the findings concerning *cyt 18* are, they are not the only instance of the implication of an aminoacyl-tRNA synthetase in mitochondrial splicing. In yeast, the *NAM2* mutation characterized by Labouesse *et al.*⁶ is of particular interest. *NAM2* was isolated as

A.N. Kolmogorov (1903–1987)

ACADEMICIAN Andrei Nikolaevich Kolmogorov, one of the most respected mathematicians in the Soviet Union, died on 20 October at the age of 84. He gained world-wide recognition for his work in probability, topology, statistical mechanics, dynamics and an enormous range of other areas. In April 1983 the Presidium of the Supreme Soviet of the USSR conferred on him the Order of the October Revolution for "his great services in the development of the mathematical sciences".

He was born on 25 April 1903 in Tambov. His mother, Mariya Yakovlevna Kolmogorova, had been delayed there *en route* from the Crimea, and died in childbirth. He was brought up by his sister Vera Yakovlevna Kolmogorova. His father, the son of a clergyman, was an agronomist. In 1920 he enrolled at Moscow University, studying history and mathematics. He retained an interest in the humanities throughout his life. His first research, done in 1922 but not published for some years, marked a spectacular debut. He gave an example of an everywhere-divergent Fourier series, a highly surprising result.

In 1939 he was elected to the Academy of Sciences, becoming secretary of the Physics and Mathematics section. Between 1964 and 1966, and since 1976, he was President of the Moscow Mathematical Society. He held many positions at Moscow State University, including Dean of the Faculty of Mechanics and Mathematics, and from 1980 the Chair of Mathematical Logic.

He published more than 300 works, which range over many different areas of mathematics, both pure and applied. In 1933 he laid the foundation for the modern

axiomatic treatment of probability theory. In the decade before 1939 he made an outstanding contribution to algebraic topology, with the invention of cohomology. In the 1940s he turned to fluid turbulence and derived his famous law on its statistical nature: the mean square difference of the velocities at two points of a fully turbulent fluid varies as the two-thirds power of the distance separating them. This result is fundamental to the idea of turbulence as a self-similar process. He advanced the understanding of the concept of entropy. In the 1950s he obtained the first result in a sequence leading to what is now known as the Kolmogorov–Arnold–Moser theorem, a cornerstone of modern nonlinear dynamics, with applications to the stability of the Solar System and to plasmas.

His interests included statistical mechanics, function theory, topology, logic, information theory, algorithms, celestial mechanics, biomathematics and mathematical linguistics. In all of them he made discoveries of lasting importance. Although his work ranged across an enormous area, it was all connected by the many links, usually surprising, which he found between apparently different topics.

His influence is best summed up in a statement made by N.N. Boglyubov, B.V. Gnedenko and S.L. Sobolev to mark his eightieth birthday: "The whole life of Andrei Nikolaevich Kolmogorov has been an unparalleled feat in the name of science. He is forever among great Russian scientists."

Ian Stewart

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a dominant suppressor of splicing deficiency arising as a result of mutations in the intronic reading frame of the fourth intron in the gene for cytochrome *b*. No other group I intron is affected by it, apparently because it functions through activation of the maturase encoded by the reading frame in the very similar fourth intron in the gene for subunit I of cytochrome *c* oxidase. The sequence of the *NAM2* gene displays similarity to bacterial aminoacyl-tRNA synthetases⁶, and more recent sequence comparisons (C. Herbert *et al.*, personal communication) indicate that it encodes the yeast mitochondrial leucyl-tRNA synthetase. As in *cyt 18*, the mutation responsible for altered splicing activity is located in the amino-terminal region of the protein.

As Akins and Lambowitz point out, the involvement of tRNA synthetases in RNA splicing has some interesting ramifications. The finding that the *Tetrahymena* rRNA intron is spliced in *E. coli*, for example, although originally interpreted in terms of self-splicing, may in fact mean that splicing is promoted by *E. coli*

tRNA synthetases and/or other proteins. Similarly, the splicing bacteriophage T4 introns in *E. coli* may also be mediated by host aminoacyl-tRNA synthetases.

From an evolutionary standpoint, it is possible that the relationship between aminoacyl-tRNA synthetases and splicing reflects a transition state in the devolvement of tasks originally carried out by RNA³. An initially self-splicing RNA may have recruited cellular RNA-binding proteins to its own ends. In many cases, gene duplication may have allowed such proteins to evolve separately. In other cases, like tyrosyl-tRNA synthetase, both functions may have been retained. □

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Olivine rheology

Creep in the mantle

S.J. Mackwell

THE deformation behaviour of minerals in the upper mantle controls convection in the mantle (which lies immediately below the oceanic and continental crust) and the coupled motion of the main crustal plates. It also controls smaller-scale processes such as mountain building, basin subsidence and motion along plate-boundary faults¹⁻⁶. It is generally accepted that, as olivine is the most abundant and the weakest of the upper-mantle minerals, the dynamics of the upper mantle will be determined by olivine rheology. Thus, an understanding of the processes that shaped the surface of the Earth requires an understanding of the creep behaviour of olivine.

In the past 10 years, much experimental effort has been focused on measuring the mechanical behaviour of olivine-rich materials under conditions that can be extrapolated to those of the Earth. Borch and Green report on page 345 of this issue⁷ a significant study of the effects of pressure and the presence of volatiles on creep in the mantle. They also develop a new criterion for the extrapolation to conditions in the Earth of experimental data obtained for the creep of olivine-rich materials.

Borch and Green address the most fundamental question in experimental rock deformation: how reliably can experimental rheologies be extrapolated to the temperature, pressure, stress and creep rate found deep in the Earth⁸? The extrapolation of experimental data has always been hampered by the narrow range of pressures available to experimentalists; Borch and Green's experiments are at higher pressures (up to 2.3 gigapascals) and have better experimental resolution than previously achieved. In particular, the use of a molten salt cell significantly reduces internal friction and improves resolution in the stress at which the samples deform at experimental creep rates in the high-pressure solid-medium creep apparatus. Borch and Green demonstrate that the strengthening of olivine caused by increasing pressure, and the weakening caused by water, can be explained by the increase and decrease, respectively, of the melting temperature. This demonstration that the strength of olivine scales with the melting temperature, independent of changes in the physical and chemical environment, considerably simplifies the extrapolation of creep data for olivine-rich materials to the Earth.

Although the extrapolation of their experimental results to the upper mantle requires that Borch and Green assume a

geotherm for the region of interest and a model for creep rate at depth, the use of experimental rheologies provides much stronger constraints on viscosities in the upper mantle than those inferred by geophysicists from crustal-plate motions and isostasy. For instance, many geophysical models assume newtonian diffusional creep in the mantle⁹, whereas experimental results suggest that upper-mantle creep involves non-newtonian dislocation creep mechanisms¹⁰; the two mechanisms yield very different viscosity profiles for the upper mantle⁹.

As no creep data have been measured directly for the spinel phase of olivine, it is unclear whether the arguments presented by Borch and Green can be extrapolated to the mantle below the olivine-spinel transition. Also, as there is insufficient data on the nature of the water-weakening effect in olivine¹¹, it has not yet been established that high viscosities in the upper

mantle necessarily imply a low content of volatiles.

Despite these limitations, however, the concept that the strength of olivine-rich materials is dependent on the melting temperature is very exciting, as it provides a mechanism for determining the effects of changes in the physical and chemical environment on the behaviour of olivine-rich materials in the Earth, simply by measuring the changes in the solidus of the material in the laboratory. □

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Evolutionary biology

Gut reactions of lysozyme

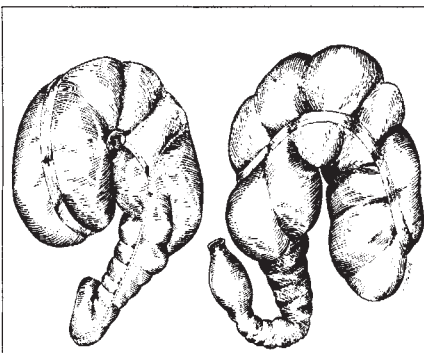
Andrew Leigh Brown

THE search by evolutionary biologists for adaptively significant amino-acid substitutions has frequently gone unrewarded. Increasingly sophisticated techniques in protein chemistry have generated a considerable level of understanding of how the sequence of many proteins is related to their function, but there are few examples where an adaptive change can confidently be ascribed to specific substitutions. Many proteins seem to have been doing the same job for a considerable period of geological time. In consequence, it is difficult

to distinguish adaptive from neutral (or nearly neutral) substitutions even in such well-documented cases as the haemoglobin molecule.

One of the most convincing arguments that a specific substitution represents an adaptive response can be made when sequence convergence has been identified in homologous proteins that have independently acquired the same new function in two different organisms. One example of this phenomenon appears on page 401 of this issue¹, where Stewart *et al.* show that lysozyme, an enzyme which has acquired a role in the digestive tract in many mammals, isolated from cow stomach and from the stomach of the leaf-eating langur monkey *Presbytis entellus*, shows sequence convergence. This result takes on a special significance when it is realized that the groups to which these two herbivorous mammals belong have independently evolved foregut fermentation as opposed to the hindgut fermentation of horses and rabbits.

The structure of the bovine stomach is well known, but the subfamily Colobinae, which includes the langurs, is also characterized by possession of a complex stomach (see figure). As in the cow, an anterior part functions as a chamber for bacterial fermentation of ingested plant matter.



The stomach of a leaf-eating monkey. Note the large, sac-like fundus or fermentation chamber and the tubular central region. Left, cranial view; right, caudal view. (From ref. 5.)

Bacteria are killed and digested in the more posterior true stomach, which is a tube-like structure. Earlier studies had shown that langur monkey stomach lysozyme and that of the cow share two adaptations to their role in the foregut — the ability to function at low pH, and resistance to proteolysis by pepsin. Stewart *et al.* have now discovered¹ that this has come about by evolution of the langur stomach lysozyme away from that of other monkeys and towards the cow sequence at five specific amino-acid sites.

The catalytic function of lysozyme is the cleavage of $\beta(1-4)$ glycosidic bonds between *N*-acetyl glucosamine and *N*-acetyl muramic acid. Together these residues form the disaccharide building blocks of the crosslinked peptidoglycan that is the chief component of bacterial cell walls. Most known lysozymes belong to one of three main classes: chicken (c-); goose (g-); or phage-type. The archetypes in the former two classes have been isolated from egg-white, and the last is encoded by bacteriophage T4. Despite the obvious evolutionary relationship between chickens and geese, there are virtually no amino-acid residues shared between c- and g-type lysozyme² and the genes encoding both may be present in many organisms³. However, almost all mammalian lysozymes belong to the c-class, although there has been considerable evolution of primary sequence, of gene copy number and of the regulation of lysozyme expression.

In most non-herbivorous mammals, c-type lysozyme can be isolated from many tissues and fluids, where it is involved in resistance to bacterial invasion. Conscription of the enzyme to a role in digestion seems to involve either a separate isozyme, as in the mouse⁴, or the loss of lysozyme activity from many of the tissues where it is normally found, as in the cow⁵. In the case of the cow this does not seem to have involved a marked increase in the rate of sequence change. (Unusually, there seems to be a considerable variation in branch length within the phylogenetic trees of c-type lysozymes isolated from mammals — it does not appear to be very "clock-like"^{1,3}.)

Study of the stomach lysozyme from the langur turns out to be more interesting in this respect. Comparison of the amino-acid sequence with those from other primate enzymes permits the construction of a type of phylogenetic tree called a Wagner network. Such trees are unrooted and at any particular site evolution can proceed in either the forward or the reverse direction. Of the 90 unrooted trees that are possible with 6 sequences, the one with the minimum evolutionary distance summed over all branches is chosen — the 'maximum parsimony' principle. Such trees permit the reconstruction of ancestral sequence at each branching point, or

node. It was therefore possible for Stewart *et al.* to allocate the changes that occurred below a node to the two descendant branches. In the case of the relatively recent node corresponding to the common ancestor of baboon and langur, this procedure gives a remarkable result. Only four amino-acid substitutions were allocated to the branch leading to the baboon, whereas ten were allocated to the branch leading to the langur. Five of these ten converged on, or occurred in parallel with, the amino-acid substitutions at the same site in the cow lysozyme sequence.

Although the maximum-parsimony assumptions underlying such constructions must always be borne in mind, the results in this case are convincing and of great interest. □

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Astrophysics

Optical flashes from a γ -burster?

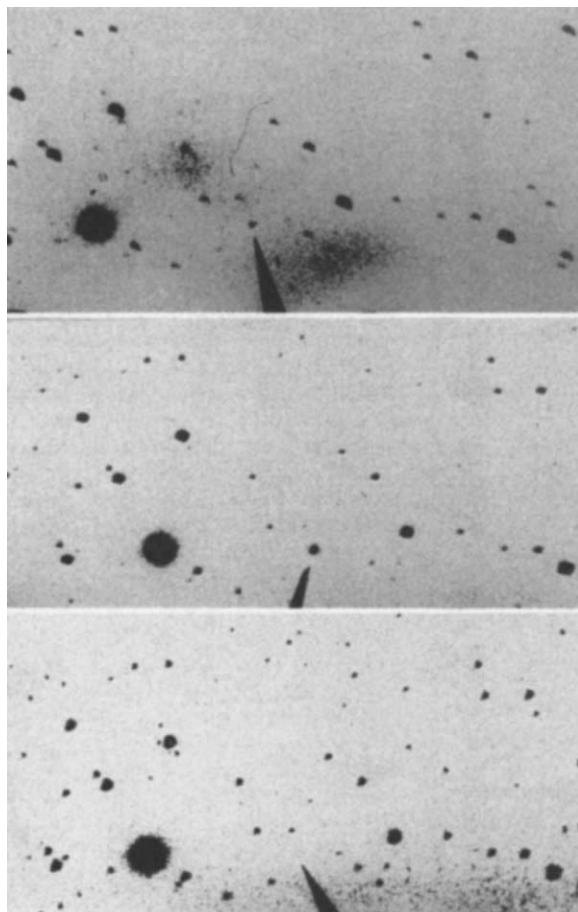
Giovanni F. Bignami

AN important new contribution to the understanding of the γ -ray burst phenomenon may have been found. René Hudec and co-workers reported at the European Regional Astronomy Meeting, held in Prague in August, the discovery on archival astronomical plates of a recurrent optical flash emitted very close to the position in the sky of a γ -ray burster,

itself not identified at other wavelengths.

Cosmic γ -ray bursts were first observed during the 1970s as an unexpected by-product of the nuclear-explosion monitoring programme in the United States. Such bursts last between milliseconds and several seconds, and some repeat at intervals of years. Soon after the discovery, a network of satellite observations was organized to yield accurate positions, sometimes with better than arc-minute resolution, for the transient γ -ray events. Other observations, such as spectra and time profiles, suggested that the phenomena were related to neutron stars, so that it came as no surprise that γ -bursters had no obvious optical counterparts. Several theories predict that a bright optical flash should accompany the γ -rays, but no detector has been flown so far that could detect one.

On the assumption, supported in at least two cases, that γ -ray bursts repeatedly come from the same object, perhaps at intervals of years, it makes sense to look for the presence of a past optical signature. Optical astronomy is fortunate in having a tremendous wealth of information in the form of photographic plates of the sky taken by generations of observers. B. E. Schaefer (*Nature* **294**, 722–724; 1981) pioneered the field of searching through such archives for evidence of past flashes at the position of known γ -bursters. Now, Hudec and colleagues (Ondrejov Observatory),



Hudec's object, indicated by the arrow, is present in two of the Sonnenberg Observatory plates (top, 28 March 1946; middle, 31 August 1946) but not in a normal plate (bottom). The bright star in the left of the plates is the 5-magnitude 104 Her, which is about 7 arc min from Hudec's object.

also using the collections of the Sonnenberg and Bamberg observatories, have systematically studied 350,000 plates.

After painstakingly searching through 28 γ -burst positions, for each of which 2,000–3,000 plates were available, they found a good candidate. In three plates, taken on 28 March 1946, 31 August 1946 and 27 April 1954, an object appears at precisely the same coordinates, where none is normally seen, even in the Palomar Sky Survey (see figure). But Hudec's object position does not coincide accurately with a γ -ray burster, being about 6 arc min from the 29 March 1979 event, the position of which is determined to within a few square arc minutes. (Laros, T. G. *et al. Astrophys. J.* **290**, 728–734; 1985). Whether this discrepancy is truly insurmountable, given the difficulty of locating bursts using different satellites, remains to be seen.

The significant point is that an optical object exists which cannot be a plate defect (there are three independent plates), a telescope problem, a vertical meteorite or an atmospheric event, an aeroplane or a satellite (all plates were taken before Sputnik was launched), and must therefore be a genuine astronomical phenomenon. Moreover, the object's images are not affected by 'trailing', caused by imperfect telescope guidance, and must necessarily have been of a short (a few seconds) duration. Thus, recurrent novae and all known types of variable stars are excluded, a conclusion supported by the large magnitude difference (> 10) between the unseen, quiescent state and the flashes.

What is the object responsible for this event? Is it also responsible for at least one γ -ray burster? However remarkable, Hudec's object cannot provide the final answer, which will come only from the contemporary detection of a γ -ray and optical flash coming from the same direction in the sky. Several ground-based efforts are now under way or being planned for coordinated optical watches. The ingenuity of these projects, however, could be compromised by the devastating increase in optical pollution caused by orbiting satellites and debris (see the recent case of the 'Perseus flasher', described in *Nature* **329**, 111; 1987).

The European Space Agency's GRASP (γ -ray astronomy with spectroscopy and positioning) satellite is also designed for γ -ray-burst astronomy. It will be able to locate γ -ray events with arc-minute accuracy and to record sensitive optical data from the same region of the sky with the same resolution. If γ -bursts occur at the expected rate during the mission, several good cases should be observed to solve the problem of their origin. □

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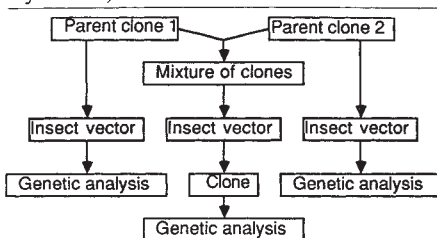
Evolution

Sex among the parasites

Paul H. Harvey and Anne E. Keymer

SEXUAL reproduction among the protozoan parasites that cause malaria and sleeping sickness in humans provides almost virgin territory for evolutionary biologists. Although it has been known for decades that malarial plasmodia go through a necessary sexual phase while in their insect vectors, sex among the trypanosomes that cause sleeping sickness is a much more recent discovery. Preliminary genetic analyses by Wells *et al.*¹ and by Walliker *et al.*² reveal unexpected features of the population dynamics and evolutionary biology of sex in trypanosomes and plasmodia, respectively.

Patterns of isoenzyme variation led to the suggestion³ in 1980 that *Trypanosoma brucei* could be both diploid and sexual. By 1986, Jenni and co-workers had



The experimental design used to demonstrate recombination among clones of *P. falciparum* and *T. brucei*. Recombinants between the two parental strains are found in clones isolated from individuals arising from vectors fed a mixture of parental strains 1 and 2. Within-strain recombination in *T. brucei* could be demonstrated only by the cloning of parasites from the separate insect-passage of each parental type.

demonstrated⁴ genetic recombination among clones of *T. brucei* fed to tsetse flies in a blood meal. It is not clear from their experiment (see figure) whether recombination occurs when vectors derive their trypanosome infections from a single source. Whatever the mechanics of genetic recombination in *T. brucei*, the system is unusual: hybrid trypanosomes have about 50 per cent more nuclear DNA than the pure-bred parental clones¹. Limited evidence suggests that some DNA is subsequently lost during successive divisions of trypanosomes in the bloodstream of their mammalian hosts^{1,5}.

Where does this DNA come from? We do not yet know. Chromosome condensation has not been observed during the trypanosome cell cycle, but it is possible to distinguish some of them during pulse-field gel electrophoresis. Hybrids seem to inherit a full complement of the smallest chromosomes from both parents, but these chromosomes inherited in double dose could account only for about 20 per cent of the additional DNA¹.

In contrast with *T. brucei*, patterns of isoenzyme variation suggest that *T. cruzi*, the cause of Chagas' disease, rarely (if ever) goes through a sexual phase even though it is diploid. Tibayrenc and colleagues discovered⁶ what seem to be several clones of *T. cruzi* that "were identified without any change over many years in various countries". There are differences in the life cycles of the two trypanosomes that may be related to their apparently divergent reproductive patterns, not least of which is the difference in intermediate host. Tsetse flies transmit *T. brucei* while feeding, but reduviid bugs pass *T. cruzi* through their faeces and the mammalian host rubs the faeces into the skin while scratching. Perhaps tsetse flies or their hosts suffer from double infections of *T. brucei* much more commonly than reduviid bugs or their hosts suffer from double infections of *T. cruzi*, leading to sexuality either evolving or occurring more readily in *T. brucei* than *T. cruzi*.

The rate at which our knowledge of sexuality among trypanosomes is changing is illustrated by one of the most recent, authoritative and popular books on immunology⁷, which provides diagrams with life cycles of the two trypanosome species and illustrates *T. cruzi* as going through a sexual phase, and the tsetse-borne *T.b. rhodesiense* and *T.b. gambiense* (subspecies of *T. brucei*) as being totally asexual.

Genetic recombination in the human malaria parasite *Plasmodium falciparum* was demonstrated for the first time earlier this year by Walliker *et al.*². In a similar protocol to the trypanosome experiments, the authors mixed two clones of *P. falciparum* and presented them to mosquitoes in a blood meal. In control experiments, they fed the pure clones to mosquitoes. Plasmodia enter the mosquitoes as haploid gametocytes. The gametocytes eventually develop into micro- and macrogametes that fuse to form zygotes, which penetrate the gut wall of their mosquito hosts and settle as parasitic oocysts from which haploid sporozoites are released. Walliker *et al.* used 1,500 mosquitoes in their experiments. The mosquitoes that had been fed the single-clone gametocytes developed about twice as many oocysts (29 per mosquito for one clone and 37 for the other) as those fed a mixture of gametocytes comprised of half from each clone (17 oocysts per mosquito). The reasons for this difference are unknown, but could result from suppression by oocysts of other oocysts with dissimilar genotypes.

Although fewer oocysts developed

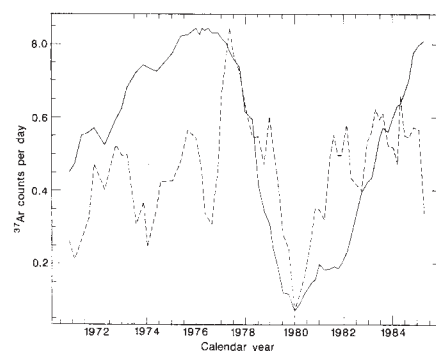
from the mixed clones, genetic markers demonstrated that recombinant sporozoites are produced in excess from mosquitoes that receive mixed clones. Assuming random mating and equal productivity per oocyst, half the sporozoites should be identical to one of the parental types. In fact, more than half those produced were recombinants. Perhaps *P. falciparum* avoids incest? If so, the explanation is unlikely to be the traditional one, avoidance of inbreeding depression. The expression of recessive deleterious alleles inherited in double dose through common descent is thought to be the main cause of inbreeding depression. For most of its life, *P. falciparum* is haploid, so most recessive genes will be expressed every generation, and shielded from selection only during the diploid oocyte stage.

If sex is maintained among most eukaryotes as a parasite-defence mechanism, as is often suggested⁸⁻¹³, then perhaps it is not surprising that parasites have joined the race by evolving sexuality to keep one step ahead of the defence mechanisms of their host(s). One intriguing question, bound to be the subject of

speculation, is why the parasitic protozoa should go through a sexual phase in their insect host whereas many multicellular parasites are sexual in their vertebrate host. One explanation might be historical: parasites normally go through a sexual phase in the host from which they first evolved. (Digenea are an obvious exception to such a theory.) In any event, the patterns and peculiarities of sex among protozoan parasites that are dependent on primary and secondary hosts beg evolutionary interpretation. □

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Average monthly sunspot number (solid curve) and the moving-averaged solar-neutrino capture rate as measured by the conversion of ^{37}Cl into ^{37}Ar (dashed curve) as a function of calendar year. The scale of the ordinate is arbitrary for sunspots, which are scaled to the same peak-to-peak range, and inverted (small sunspot number at top). (From refs 1 and 9.)

the correlation by nine orders of magnitude. A previous calculation⁸ showed that cosmic-ray secondaries that decay in flight to produce high-energy neutrinos are insufficient to account for the suggested effect by at least three orders of magnitude. The inconstant SNU's are unlikely to be produced by acceleration of energetic particles in the solar magnetic field as this would conflict with accepted ideas about the solar corona and solar flares⁹.

My own view is that the suggested correlation is a rare fluke, although many disagree. Our statistical analysis⁹ shows that even with the most optimistic interpretation of the experimental errors a correlation as strong as that in the real data is found in 2 per cent of the cases of randomly shuffled time sequences of that data. Almost every event of interest in life, the circumstances in which we first meet our spouses for example, has an *a posteriori* probability of less than 2 per cent. If one correlates the time sequences of enough physically unrelated phenomena, then some will appear to be correlated at the 2 per cent significance level or even higher.

What must be done? Observations made with the chlorine detector during the maximum of the next sunspot cycle (especially in 1990 and 1991) will test whether the correlation does exist. Ray Davis and I have a bottle of champagne wagered on the outcome. □

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Solar physics

Neutrinos and sunspots

J.N. Bahcall

THE surprising discrepancy between the calculated and the observed rate of solar neutrino captures in chlorine (SNU's) is a long-standing puzzle that has spawned many imaginative scientific explanations and several science-fiction novels. The Japanese proton-decay detector Kamio-kande, which was converted into a solar-neutrino detector just in time to make the first ever observation of supernova neutrinos, has recently confirmed the solar neutrino discrepancy. R. Davis has pointed out¹ that the neutrino capture rate is apparently inversely correlated with sunspot activity (see figure). But on page 353 of this issue², A. de la Zerda Lerner and K. O'Brien present calculations that show one plausible explanation of this modulation is not correct.

What is the significance of the suggested correlation? If it is real, then the observed capture rate does not reflect the rate of neutrino production by nuclear fusion in the solar interior because the characteristics of the solar interior are expected to vary significantly only on the timescale of nuclear burning, which is a billion years or so. Then models based on resonant neutrino oscillations in matter³⁻⁵, by which neutrinos switch back and forth between detectable and undetectable types, would have to be revised, disappointing many physicists who have been delighted by the oscillation explanation, which uses

theoretically attractive values of the mass differences and mixing angles of different neutrinos. Nature might have failed to embody a beautiful theory.

Several Soviet theoretical physicists have proposed^{6,7} that the correlation between SNU's and sunspots is caused by an unexpectedly large electric or magnetic moment of the neutrino. The correlation reflects, on this hypothesis, the changing structure of the solar magnetic field, which varies with epoch in the sunspot cycle and which could convert detectable (left-handed) neutrinos to sterile (right-handed) neutrinos.

Another, more conventional, idea is that cosmic rays or their secondaries, which produce neutrinos in the atmosphere, cause the correlation of sunspots and neutrino captures: cosmic rays are modulated by the solar wind encountered on the way to the Earth, causing the cosmic-ray intensity at the Earth's orbit to wax and wane in inverse correlation with solar activity. In this issue², de la Zerda Lerner and O'Brien describe a careful calculation of the neutrino emission from positrons produced by cosmic rays as they strike the Earth's atmosphere. They calculate the spontaneous decay rate of β -unstable isotopes produced when secondary particles collide with atmospheric ions. The calculation shows that the positron-neutrino emission falls short of explaining

Nuclear installations and childhood leukaemia

SIR—Forman *et al.*¹ assert that on present knowledge it is difficult to attribute their findings of a raised incidence of child leukaemia around nuclear installations to ionizing radiation. This conclusion is based on the premises that leukaemia risk per unit dose has not been underestimated and that computed population doses are far too low to account for the increase. Both assumptions are widely held among cancer epidemiologists and radiobiologists, but remain open to question.

In the case of Sellafield, the dose calculations of the National Radiological Protection Board (NRPB) were characterized by profound uncertainties, particularly in the case of alpha-emitters such as Pu and Am^{2,4}. The dosimetric models were essentially those recommended in ICRP30 (International Commission on Radiological Protection) for adult workers⁵. For children and the fetus the NRPB made rudimentary alterations to take into account physiological rather than metabolic changes, essentially changes in organ dimension and mass^{6,7}. ICRP 30 states clearly: "The Commission does not recommend the use of the data and models described in this report to estimate committed dose equivalent to members of a population, for example from radionuclides in the environment, by adjusting solely on the basis of differences in mass of organs or magnitude of intake"⁸. Yet, because of the uncertainties, this is precisely what the NRPB was in large measure forced to do.

Consequently, dose and therefore risk was taken to decrease in reciprocal relation to the rapidly growing body of the fetus and young child. For an alpha-emitter lodged in the skeleton, tissue mass is relatively unimportant, and at the cellular level alpha-dose averaged throughout the tissue appears biologically almost meaningless. To calculate the risks of alpha-induced leukaemia it would be necessary to know the exact location of leukaemogenic cells, yet this information is simply not known⁹. For example, lymphatic leukaemia has not been induced in the lungs of dogs or mice, but it has in rats; Sir Edward Pochin of the NRPB has remarked in this regard: "I do not know whether we are closer to the dog, the mouse, or the rat in terms of lymph-node behaviour"¹⁰.

Risk factors are similarly disputed, and the study¹¹ cited by Forman *et al.* in support of their conclusions is but one contribution to an extensive controversy over this question. Uncertainty over alpha dose-response is particularly acute¹².

Because there is judgement as well as technical evaluation of the data, it is inevitable that there will be dissent from the

values put forward; there is no evidence to disprove the ICRP risk factors, but neither is there evidence to verify them¹³.

Given that the pattern of child lymphatic leukaemia around nuclear installations is most unlikely to have occurred by chance, that it could indeed have been caused by ionizing radiation, and that we know of no other likely cause, it would seem at least prudent to give the benefit of the doubt to the local populace, and not, as it has been, to the nuclear industry.

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Why cystic fibrosis is on the increase

SIR—In a recent News and Views article, Rotter and Diamond¹ described four principles by which recessive alleles responsible for inherited diseases may be maintained at high frequencies. The most common deleterious recessive among Europeans, that which causes cystic fibrosis (CF), seems to owe its high frequency to yet another cause.

In 1965, Danks *et al.*², working in Melbourne, reported indications of increased fertility associated with CF. Grandparents of patients, unaware of their carrier status, were found to produce on average 4.75 live offspring, of which 3.42 themselves reproduced, whereas matched control couples produced only 4.13 offspring of which 3.30 had children. This finding was confirmed by Knudson *et al.*³, who found that grandparents of Californian patients had produced on average 4.34 children, compared with the 3.43 of controls, 3.86 and 3.02 respectively surviving to adulthood. Danks *et al.* also recorded a striking excess of males (1.54:1) among sibships containing CF patients.

These analyses provide no indication whether the effects of CF on fertility and sex ratio operate through male or female parents, but my colleagues and I found⁴ that the families of blood-related uncles of British CF patients, half of whom should be carriers, averaged 2.78 children, while

aunts' families, averaged 2.19, and unrelated, matched controls 2.35. Although these differences are not statistically significant, uncles were the only group to exceed their originally intended family size and not want more children. No children in any group had CF. The ratio of male to female children in uncles' families was 1.78, while that in aunts' was 1.19 and in controls was 1.00, representing a significant excess of boys in uncles' families.

Taken together these data indicate that the CF allele increases the fertility of men who carry just a single copy, but this relates to the production of male offspring only. Knudson *et al.* calculated the rate of increase of the CF allele as around 0.8 per 10,000 per generation, but their calculations do not include the contributory effect of sex ratio, which ensures overrepresentation of the more fertile carrier males in the next generation. In the modern world we must add to this the contribution of CF homozygotes who can now survive to maturity.

The CF allele arose perhaps 23 generations ago⁵ and has been increasing in frequency ever since. At present about one in every 25 white people are carriers. We can expect this proportion to be very much larger in future generations.

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Cross-talk between PKC and cyclic AMP pathways

SIR—In a recent letter to *Nature*, Yoshimasa *et al.*¹ show that on exposure of frog erythrocytes to phorbol ester, phosphate (³²P) is incorporated into the catalytic unit of adenylate cyclase, and there is a twofold increase in cyclase activity of detergent-extracted cell membranes. In a separate experiment, the authors show that phorbol-ester-activated protein kinase C (PKC) can induce the phosphorylation of bovine brain adenylate cyclase. They conclude that kinase C can activate the cyclic AMP pathway by phosphorylation of the catalytic unit of adenylate cyclase. The authors do not, however, present any quantitative relationship between the extent of adenylate cyclase activation and that of enzyme phosphorylation in their experiments. It is likely that the yield of adenylate cyclase solubilization is higher in the phorbol-ester-treated membranes and therefore that higher activity is observed in the detergent extract. The authors also do not show that

the phosphorylation of the purified bovine brain catalytic subunit (Fig. 3 of ref. 1) leads to its activation.

In intact cells where both the cyclic AMP pathway and the PKC pathway have been shown to function, it has been shown that the link between the two pathways is most probably G_i (the inhibitory GTP protein of cyclase)². Potentiation of adenylate cyclase by the PKC pathway results from the phosphorylation of G_i , thus relieving the inhibitory tonus on adenylate cyclase^{2,3}. The effect of phorbol esters is quantitatively similar to that of pertussis toxin². Yoshimasa *et al.*¹ did not examine the G_i component in their system.

Another major consideration is the possibility that the phosphatidylinositol-bisphosphate breakdown pathway and the adenylate cyclase pathway interact through the mobilization of Ca^{2+} and not only through the activation of PKC. The ample evidence on the activation of adenylate cyclase by Ca^{2+} -calmodulin, both in the central nervous system and peripheral tissues (for example, ref. 4) should alert us to the possibility that inositol trisphosphate-induced mobilization of Ca^{2+} could lead to activation of calmodulin-sensitive adenylate cyclase. Attractively, the mode of activation is transient, the property of a true signal. Simultaneous activation of PKC on phosphatidylinositol bisphosphate breakdown can subsequently result in the inhibition of calmodulin-dependent adenylate cyclase⁵, providing long-term inhibition.

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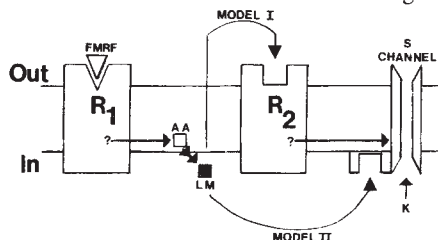
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Second messengers or second agonists?

SIR—In the recent article by Piomelli *et al.*¹, an unidentified lipoxigenase metabolite was described as a "second messenger" in presynaptic inhibition of *Aplysia* sensory neurons by FMRFamide. By definition, a second messenger is the first intracellular signal generated in response to an intercellular primary messenger, such as a hormone or neurotransmitter (model II). This definition raises two important issues: the number of steps between activation of FMRFamide receptors and production of active lipoxigenase mediators; and the distinction between intracellular and extracellular targets of the mediators. An alternative hypothesis, (model I) consistent with the authors' own

data and for which there are ample biological precedents, is that a lipoxigenase metabolite, possibly the downstream product of another messenger pathway, may act at the cell surface through its own receptor as a second agonist (see figure).

On the first issue, the first documented products of stimulation are 5- and 12-HETEs (5- and 12-hydroxyeicosatetraenoic acids) derived from arachidonic acid, suggesting that a spectrum of candidate mediators are generated by a FMRFamide-regulated biochemical cascade. Identification of the signal among the various products was undertaken by comparing the actions of synthetic eicosanoids to that of FMRFamide. Both arachidonic acid and lipoxigenase pathway-derived products mimic the action of FMRFamide in some, but it must be emphasized, not all respects. However, because the results come exclusively from experiments using extracellular application of these agents, the argument for an essential intracellular action of a lipoxigenase product must be carried by the indirect, pharmacological evidence. One reagent, *p*-bromophenacyl bromide, is described as a "phospholipase A2 or diacylglycerol lipase" inhibitor, but is in fact a non-specific histidine or carboxylic acid-modifying agent, and its specificity and ability to cross the plasma membrane in whole living cells is unknown. A selective effect of this reagent on lipid lipases would have been more convincing if



responses to the products of lipase action (for example arachidonic acid, 12-HPETE (12-hydroperoxyeicosatetraenoic acid) were unchanged after treatment. NDGA (nordihydroguaiaretic acid) is also both a cyclo- and lipoxigenase inhibitor in mammalian neutrophils², and may have similar complexities in *Aplysia* neurons. The data therefore are necessary but not sufficient to establish that the action of FMRFamide on the S channel is directly due to intracellular activity of a lipoxigenase mediator.

The functions and modes of action of eicosanoids have been examined in most detail in vascular and immune cells, which have cell-surface receptors for both cyclo- and lipoxigenase-derived metabolites^{3,4}. There is little evidence for intracellular targets for these classes of compounds.

In the platelet, activation of several distinct receptors initiates a cascade of events, including the production of eicosanoids such as thromboxane A2. For example, platelet-activating factor (PAF) stimulates the hydrolysis of inositol

phospholipids and evidence indicates that arachidonic acid is also released. Furthermore, thromboxane A2 amplifies the response to PAF by acting on its own surface receptor to stimulate further inositol lipid hydrolysis. If platelets can be used as a model for *Aplysia* neurons, then the second messenger that ultimately causes arachidonic acid release and its subsequent metabolism to active products remains unknown, and a lipoxigenase metabolite may act extracellularly through its own receptor to control channel activity.

We feel caution is required in cause-and-effect interpretations of the application of non-selective lipase-blocking agents and broad spectrum cyclo- and lipoxigenase inhibitors to intact cells.

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Anucleolate mutant due for re-examination

SIR—I was intrigued to read, in the account of recent research on the structure and function of the nucleolus by Gwyn Jordan¹, the current idea that the fibrillar centres represent the pre-initiation complexes of ribosomal RNA genes. My interest stems from the fact that, in 1965, I described² similar structures in the nuclei of the anucleolate mutant of *Xenopus* and suggested, on ultrastructural grounds, that they might be homologous with what are now referred to as fibrillar centres. If this interpretation is correct, these regions obviously can exist in the absence of ribosomal DNA. I would be curious to know whether anyone has recently examined this mutant with the newer techniques summarized by Jordan. If not, such a re-examination might help to resolve this interesting question.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

Wild possibilities

M.S. Swaminathan

Gene Banks and the World's Food. By D.L. Plucknett, N.J.H. Smith, J.T. Williams and N.M. Anishetty. Princeton University Press: 1987. Pp. 247. \$35, £22.

NINETEEN eighty-seven is the centenary of the birth of Nikolai Ivanovich Vavilov, to whose memory this book is dedicated. Speaking at the Sixth International Congress of Genetics held in 1932 at Ithaca, New York, Vavilov stressed that "the growing needs of civilized man and the development of industry make the introduction of new plants necessary. The vast resources of wild species, especially in the tropics, have been practically untouched by investigation".

During the past 50 years, interest in the search for new genes has grown enormously among plant breeders. The reasons are, first, the threat of genetic erosion arising from the destruction of natural ecosystems such as tropical forests; second, the replacement of thousands of native cultivars with a few high-yielding ones, thereby increasing genetic vulnerability to pest epidemics; and third, the new opportunities opened up by tissue culture and genetic engineering for moving genes across sexual barriers.

This interest has been reflected in the growing global efforts to establish *in situ* (biosphere reserves) and *ex situ* (gene banks) systems for conserving plant genetic resources. The establishment in 1974 of an International Board for Plant Genetic Resources (IBPGR) by the Consultative Group on International Agricultural Research (CGIAR) was a major step in promoting the collection and conservation of representative samples of naturally occurring genetic variability in economic plants. This book contains a detailed account of the work in progress.

In the past, public and political interest (and consequently financial support) in plant conservation work have not been commensurate with the size of the tasks to be dealt with. Politicians readily understand soil erosion, because it stares them in the face, but not gene erosion, which is invisible to the naked eye. Today, we find that things have changed, as is evident from even a casual perusal of this book. For example, enthusiasts for the conservation of wild fauna now find that it is equally important to conserve wild flora,

inasmuch as their fates are interlinked, and the World Wildlife Fund has thus changed its name to the Worldwide Fund for Nature. Nonetheless, some of the principal habitats that provide niches for biological diversity are fast being destroyed. The International Union for the Conservation of Nature and Natural Resources estimates that more than half of the world's tropical forests have dis-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Banking on the future — the turnip Brassica campestris grown under 24-hour sodium illumination, treated with a mutagenizing agent and selected for the quickest-flowering lines.

appeared since the turn of the century and that the current rate of deforestation exceeds 11 million hectares a year.

The subject of *ex situ* conservation of plant genetic resources has recently become a controversial topic for reasons more related to commerce than concern for sustainable food security. Thus it is now common to see articles with titles such as "Seeds and Genetic Imperialism" in newspapers and periodicals. Among other events, two developments have triggered a process in which genetic conservation work is increasingly taking on commercial and political overtones.

First, many developed countries have adopted legislation for plant breeder rights (PBR), leading to patents for new varieties which are administered through the International Union for the Protection

of New Varieties established in 1961. That has led to the apprehension that plant genetic resources collected in developing countries for storage in developed countries will become a *private* resource and may not be freely available to the countries where the collections were made. This fear is compounded by the suspicion that many chemical firms, which are now entering the plant breeding field, may not incorporate resistance to those pests and diseases for which they have developed effective chemical control agents.

A recent example of the trend towards privatization of plant breeding research in developed nations is the decision of the British government to sell its Plant Breeding Institute at Cambridge, which during the past 75 years has been the origin of

many outstanding achievements, both in the theoretical and practical aspects of crop breeding. In addition, it has been a major training institution for plant breeders from developing countries. If this trend continues, developing countries whose genetic resources have gone into the making of modern varieties may have access to those varieties only on commercial terms.

That leads to the second area of concern. Although the genes needed for a dynamic plant-breeding programme are found mainly in primitive cultivars and wild species in the tropics and subtropics, the gene banks where they are preserved for current and future use are located largely in developed countries. The authors of this book point out that the major exception is the gene banks established by the international agricultural research centres (IARCs) funded by CGIAR and those promoted

by IBPGR in developing countries. The International Rice Germplasm Center of the International Rice Research Institute at Los Banos in the Philippines, for example, has over 80,000 accessions of wild and cultivated rice strains and allied species.

The IARCs regard themselves as the custodians — not the owners — of the genetic collections conserved in their gene banks, and thus treat the collections as a *public* resource to be made available to all researchers working to improve a particular crop. Although doubts over the legal status of the IARC collections raise questions about their continued non-commercial availability, the UN's Food and Agriculture Organization has recently formulated several legal options to ensure that the genetic collections in different parts of the world remain a common

heritage of humankind. Many countries have also subscribed to an International Undertaking on Plant Genetic Resources, formulated by FAO in 1983 to promote the free sharing of plant genetic resources. Such developments have resulted in a movement to promote "Farmers' Rights" and confer on farm families in the tropics and subtropics benefits similar to those given to plant breeders of developed nations under the PBR legislation. For it is largely farmers' efforts, in conscious and unconscious selection and maintenance of genetic diversity, that have led to the availability of such valuable genetic material to the plant breeders of developed countries. For the first time in agricultural history we are confronted with terms such as "Breeders' Rights" and "Farmers' Rights", as though the interests of breeders and farmers are somehow antagonistic.

In this context, *Gene Banks and the World's Food* is a timely publication. The authors are experienced scientists and have assembled a wide range of useful information and data covering all aspects of germplasm collection, characterization, conservation and use. They rightly stress that the availability of genetic variability by itself is no insurance against genetic vulnerability to pests and diseases, unless

trained plant breeders use that variability effectively. Gene banks are of little value unless we know what genes they contain. The authors also refer to the new opportunities opened up by tissue culture and molecular biology for *ex situ* conservation, particularly of clonally propagated material. Genetic engineering techniques have enhanced the value of wild species and primitive cultivars, and DNA-transfer techniques are being rapidly refined.

The book will serve as an excellent source of information, particularly about the work being done by IARCs. The authors concentrate largely on the scientific aspects of conservation, and make only passing reference to matters relating to ownership, unrestricted access, and the long-term implications of plant patent rights in a two-page section titled "Seeds of Discord". A companion volume dealing with these items in an equally factual and lucid manner would be valuable. The subject of genetic resources is no longer the exclusive concern of scientists, as it was in 1932 when Vavilov first advocated plant-collecting expeditions. □

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Summing it up

C.J.S. Clarke

Encyclopedic Dictionary of Mathematics, 2nd Edn. The Mathematical Society of Japan, editor-in-chief Kiyosi Itô. MIT Press: 1987. Four volumes, pp.2,120. \$350, £250.

THE mathematical physicist is a jackdaw, constantly picking up bits of mathematical information; and for many of us the main source of such trinkets has long been the *Encyclopedic Dictionary of Mathematics* (EDM). Such tools of research are habit-forming, and I for one would now be lost without the *Dictionary*. With the appearance of the second English edition (the original is in Japanese), any important part of main-stream mathematics, up to 1985–1986, can be located in a very comprehensive index, and pursued through an excellent system of cross-references. In many cases the articles give all the information needed; in others, one learns enough to know where to go next and what to look for.

The new edition is a revision, rather than a rewriting, of the first, with expansion of the material by about half and reorganization of the topic-headings. A major improvement is the provision of references to English-language textbooks, in place of the original Japanese ones. The

presentation has been made even clearer, particularly in the index. Much of the material, however, including the extensive tables in the appendices, has been reproduced unchanged (with some minor misprints preserved).

Many of the additions are predictable, and a comparison of the differences between the editions provides an interesting view of recent developments in mathematics. Differential topology was awarded only one page in the first edition; the topic now has nearly ten, and includes an account of the remarkable work of M.H. Freedman and S.K. Donaldson on 4-manifolds, whose impact on physics will probably be great in years to come. The article on the four-colour problem, which remains one of the few great mathematical problems that can be explained to a layman, has been rewritten, following its solution in 1976 by Haken, Appel and Koch. Some of the more fashionable aspects of mathematics are represented: fractals are there, with the definition of Hausdorff dimension (surprisingly omitted from the first edition), as are solitons, though not Bäcklund transformations. Renormalization group ideas enter with a new article, as does singularity theory, with accompanying tables. But one detects a certain puritanism in selection: the Monster group, though briefly mentioned, remains anonymous.

Away from the traditional centre of mathematics, and in applications, more

controversial decisions had to be made as to what to include. One must applaud the addition of a new section (at the top level of classification) partly on computer science; but its scope is limited by also including combinatorics, coding theory and complexity theory, as well as mathematical models in biology. The treatment of all these topics is thin: one might have hoped for something on public key encryption and stochastic approaches to complexity theory (I could not find either), and more on Chaitin complexity and linguistic issues in computer science. But the section on automata, painfully inadequate in the first edition, has now been adequately rewritten.

There are sections on the history of mathematics (mainly organized bibliographically), on statistics and on operations research; and an extensive section of articles on mechanics and theoretical physics. This last includes a few pieces of a different character, sometimes non-mathematical, surveying an area of mathematical physics and aiming, one suspects, more at giving background information to the mathematician than detailed information for the physicist. These cover, for example, field theory, elementary particles and general relativity, and are well written within their limited scope.

Some of the more recent needs of the theoretical physicist have been met. The worker in string theory will find a modest enlargement of the material on Teichmüller spaces, which is now all collected in one article under that name. But a notable omission is super-mathematics: the graded versions of Lie algebras, Lie groups, manifolds and so on that have become so crucial for quantum gravity and string theory. The nearest one gets to these is a brief mention of Hopf co-algebras. Are the editors primly upset by appellation of 'super' to all these fields, or have they occult information that they will prove to be a passing craze?

A competitor to EDM is on the horizon, in the form of *The Encyclopaedia of Mathematics*, an updated and annotated translation of the Soviet *Mathematical Encyclopaedia*; the first volume has just been published by Reidel, and will be followed by another nine. As far as I can tell from the sample pages so far released (I have not seen the original), the coverage could be greater and the approach more careful pedagogically than that of EDM: the Soviet encyclopaedia explains, where EDM states. But for those who prefer a bird in the hand rather than waiting for the completion of publication of the Soviet work in 1990, the EDM is an essential buy for every library and for many private bookshelves. □

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Muddy waters

Eville Gorham

Acidification of Freshwaters. By Malcolm Cresser and Anthony Edwards. Cambridge University Press: 1987. Pp.136. £19.50, \$34.

THE acidification of freshwaters is a controversial topic. The phenomenon is viewed by many as a consequence of acid deposition from the atmosphere, and by others as largely the natural result of ecosystem development, often influenced by human land-use. In their book, Cresser and Edwards attempt to take a balanced view, examining processes by which ecosystems acidify naturally and ways in which human activities — changes in land use, atmospheric emissions of sulphur and nitrogen oxides — influence acidification. The authors stress the complexity of the subject, and include useful references to infrequently cited British work, helpful notes on methodology and suggestions for future research.

So short a book inevitably has drawbacks. The history of the subject is confined to a mention of the pioneering work of Robert Angus Smith. Modelling is accorded two pages and a citation of the 1984 book edited by J.L. Schnoor (*Modelling of Total Acid Precipitation Impacts*). Target loadings to protect susceptible freshwaters from acid deposition are not considered, and important studies at the Experimental Lakes Area in northwestern Ontario, dealing with subjects such as the countering of acid deposition by

generation of alkalinity within lakes, and the very earliest stages of biological response to experimental lake acidification, are not mentioned.

The geographical extent of freshwater acidification, and its empirical relationship to bedrock and soil type and the severity of acid deposition, are not examined, although such considerations are helpful in assessing the factors involved in acidification. For instance, if natural acidification and land-use changes are of widespread importance in the acidification of clearwater streams and lakes, why is it largely restricted to regions where sensitive substrates are subject to precipitation with a mean pH of less than 4.8? In this connection it is nowhere explained that waters naturally acidified by organic acids produced during the decomposition of plant detritus, for instance the porewaters of surface organic soils or streams and lakes draining peatlands, can be distinguished by their yellow-brown colour and high concentration of dissolved organic matter from clear waters acidified by atmospheric deposition of strong mineral acids. The major role suggested for sodium chloride of marine origin in natural acidification is questionable.

A short book for a broad audience cannot cover everyone's favourite topics, but at least some of those mentioned above deserve attention in any account of fresh-water acidification. Despite these deficiencies, Cresser and Edwards have provided a useful introduction to the subject. □

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Turning the tables for chemists

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Calculated Molecular Properties of Polycyclic Aromatic Hydrocarbons. By Ronald A. Hites and William J. Simonsick, Jr. Elsevier: 1987. Pp. 272. Dfl. 260, \$126.75.

THE polycyclic aromatic hydrocarbons are a large and important class of chemical carcinogens which enter the environment through the incomplete combustion of organic materials. Chromatographic techniques for the separation of complex mixtures of these compounds are quite well developed, although identification of the individual components is often tedious.

One solution suggested by the authors of the present volume is based on an empirical correlation between experimentally determined data from charge exchange chemical ionization mass spectrometry and ionization potentials calculated through quantum mechanics. *Calculated Molecular Properties of Polycyclic Aromatic Hydrocarbons* appears to be an outgrowth of that effort. It is not a textbook of any kind, but simply a tabulation of theoretical data for benzene and 270 polycyclic aromatic hydrocarbons obtained using the MNDO semi-empirical molecular orbital procedure.

Unfortunately, the introduction is a mere four pages in length and provides only a superficial description of the tabulated quantities. However, the data themselves are conveniently laid out. Each compound is described on a separate page by beginning with the preferred chemical abstracts name, followed by the molecular formula, molecular weight and CAS registry number. Next come the calculated heat of formation, total, electronic and nuclear energies, the energies of the highest occupied and lowest unoccupied molecular orbitals and the dipole moment. Calculated geometric data are not given, although a scale line-drawing of each structure is provided together with a table of calculated atomic charges. Two non-quantum mechanical properties related to the overall molecular shape are included; both are claimed to be useful predictors of chromatographic behaviour.

This is not a book for the general reader. But it is easy to think of situations in which some of the tabulated quantities could be of value to researchers in this area. □

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Grim legacy — the 'becquerel reindeer', a freezer of radioactive meat in a slaughterhouse in Swedish Lapland. Meat from reindeer feeding on lichen contaminated with caesium-137 from the Chernobyl cloud contained up to 16,000 becquerels per kilo; the maximum radiation normally allowed in Sweden is 300 becquerels per kilo. The photograph is taken from *At Work in the Fields of the Bomb* by Robert Del Tredici, a collection of photographic images on the theme of nuclear weaponry and installations. Published by Harper and Row, the book costs \$35 (hardback) \$15.95 (paperback).

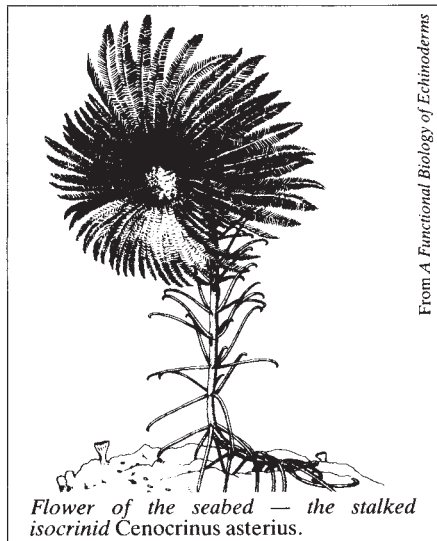
Stars of biology

Dai Roberts

A Functional Biology of Echinoderms. By John Lawrence. Croom Helm, London/ Johns Hopkins University Press, Baltimore: 1987. Pp340. £40, \$56.50

PENTAMERY, the water vascular system and a distinctive skeleton mark echinoderms apart from all other animal phyla. The nature and origin of these features remain puzzles for all students of the group, and for biologists more widely. By bringing together earlier comparative structural information and the more recent, exciting developments in echinoderm biology, John Lawrence brings a new perspective to these problems.

Without labouring details of structure, which are assumed and summarized in an invaluable appendix, Lawrence rapidly introduces the reader to present interpretations of the interrelationships and



Flower of the seabed — the stalked isocrinid *Cenocrinus asterius*.

diversity of echinoderm groups. Chapters deal with nutrient acquisition, maintenance and reproduction, central features of modern functional biology.

Details of feeding processes in different taxa form the basis for a comparative study of feeding efficiencies. Lawrence shows how enhanced feeding by structural elevation of the feeding arms of stalked crinoids is paralleled by functional elevation in the stalkless comatulids. Similarly, the orientation of suspension-feeding structures and intraspecific aggregations, which are particularly dense in some groups, may enhance particle capture.

The great adaptability of animal structure is particularly well illustrated by echinoderms, which show repeated adjustment of a relatively narrow suite of characters to diverse functions. Tube feet may be used for respiration and feeding as well as for locomotion; echinoid spines may have roles in locomotion and feeding as

well as for defence; mutable collagenous tissue may maintain feeding posture in crinoids or provide defensive rigidity in holothuroids. The enigma of the apparent absence of a circulation based on a single system common to all groups is probably yet another aspect of this structural adaptability, with different systems taking on a circulatory role in different groups. Lawrence documents many of these aspects of echinoderm biology, but more

importantly assesses them in terms of their costs and benefits.

The illustrations are good and the writing clear, and with over 600 references the book puts the available information into a helpful perspective. It will be an important resource work for students of echinoderms for the next decade. □

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Drilling for victory

Joe Cann

The Superdeep Well of the Kola Peninsula. Edited by Ye. A. Kozlovsky. Springer-Verlag: 1987. Pp.558. DM248.

IN May 1970, the SG-3 borehole was spudded into the Precambrian crust of the Kola Peninsula in Arctic Russia in an ambitious attempt to drill through thick Proterozoic sedimentary and volcanic rocks, to cross the Conrad discontinuity in the Earth's continental crust and to sample the lower crust in place. After ten rig-years of drilling, using two rigs, a total depth of 11.5 km had been reached when this book was published in Russian in 1984, and further penetration was planned.

Here we have, translated into English, a full account of both the science and the technology of the project. It was both a great success and a mild failure — a failure because the Conrad discontinuity did not appear as expected. This was not surprising because over the past ten years evidence has become stronger and stronger that the Conrad was simply an artefact of the seismic processing methods used in classic seismic studies, and never did exist. The graphic proof comes from the log of this hole, and down with the Conrad discontinuity clatters the cumbersome apparatus of Sial and Sima, of granitic and basaltic layers in the continental crust, of the now extinct idea that oceanic crust continued beneath the continents. So even in failure the project had positive virtues.

What about its successes? First, there is the mere fact of drilling such a hole. Part 3 of the book discusses the drilling technology used and the problems that had to be overcome as the hole went deeper and deeper into rock more and more stressed by the overlying load. Turbo drills were deployed and in turn allowed use of light alloy drill pipe. High-temperature drilling muds were developed. Problems of rock fracturing became more intense. The discussion here is very detailed and most instructive, especially for those of us involved with scientific drilling elsewhere.

Part 2 is concerned with downhole logging of the sequence and with laboratory physical studies, and Part 1 with the

geological aspects of the drilling. In places the usual profound irritations of Russian texts emerge. Nowhere, for example, in the section on geothermal measurements, is any table or figure of temperature with depth given — I still do not know the temperature at the bottom of the hole. Nor, in the section on seismic velocity logs, is there any scale on the numerous plots of V_p and V_s downhole, though here you do have a table to guess from. Sloppiness? Lack of concern for the reader? Or is there some profound necessity in keeping such information entirely obscure?

Most exciting of all of the results reported during the drilling of the well were that water flowed into the hole deep in the section, and hydrocarbons were found at depth in the gneisses. There is good reason to believe that the lower continental crust should be both dry and free of hydrocarbons, and I turned to the chapters on organics and water with great interest.

Unfortunately, both phenomena are not well documented. There were clearly problems in sampling fluids from the wells, because a formation tester could not be deployed deep in the section, but the lack of detail in the accounts does not allow a judgement of just how good or bad the samples were, or how liable to contamination. In the end I felt mildly baffled again by Russian obscurity. So the book is patchy — in places fascinating, in others irritating — but it documents what must be considered a spectacular achievement.

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New in paperback

- *The Mind of a Mnemonist* by A.R. Luria. Publisher is Harvard University Press, Price is \$7.95, £6.25.
- *The Beginnings of the Nobel Institution* by Elisabeth Crawford. Publisher is Cambridge University Press, price is £9.95, \$16.95. For review see *Nature* 314, 619 (1985).
- *The Search for Solutions* by Horace Freeland Judson. Publisher is Johns Hopkins University Press, price is \$10.95, £6.65.
- *Actual Minds, Possible Worlds* by Jerome Bruner. Publisher is Harvard University Press, price is \$7.95, £6.50.
- *The Physicists: The History of a Scientific Community in Modern America* by Daniel J. Kevles. Publisher is Harvard University Press, price is \$12.95, £10.25.

Fruits of northern friendship

Nordic cooperation is a natural response to the small size of the countries in the region and their natural affinities. It should not be allowed to impede partnerships that are fruitful for research.

If the strongest advocates of Nordic cooperation had their way 'Norden' would be a familiar term. It describes a group of countries that have much in common in history and culture. The fact that Norden remains a word more suited to quiz shows than geography books does not mean that cooperation is non-existent in the Nordic area. In fact, it has been growing since the Second World War and has become institutionalized in a number of ways. In formal terms, cooperation has been marked by the establishment of the Nordic Council in 1953 and the Nordic Council of Ministers in 1971. Among the results are that Nordic nationals can readily work and settle in any Nordic country. For scientists there are also a variety of cooperative organizations and programmes. Are these beneficial for Nordic science?

While 'Scandinavia' usually refers to Sweden, Denmark and Norway, the Nordic region includes two other sovereign states and three territories with self-rule. Of the states, Sweden has the largest population (8.4 million) and Iceland the smallest (240,000). Denmark, Norway and Finland each have a population of between 4 and 5 million. The self-ruled Danish territories of Greenland and the Faeroes and the Finnish Åland Islands each have fewer than 50,000 inhabitants.

Current Nordic cooperation has been preceded by various historical combina-

tions of political union, and a few of conflict, between the member states. In particular, Denmark, Sweden and Norway once formed the Kalmar Union (1397–1523). Thereafter Sweden was often at war with Denmark and Norway until 1660 when the present boundaries of the three countries were agreed. Norway and Sweden, were united under one monarch from 1814 to 1905 (which is why the Nobel Prize for Peace is even now awarded through the Norwegian parliament). Finland, freed from Russian rule in 1917, and Iceland, freed from Danish rule in 1944, are the youngest Nordic states.

Denmark, Iceland, Norway and Sweden were the founder members of the Nordic Council; Greenland was last to join in 1984. The council is the main forum for cooperation between the parliaments and governments of the Nordic countries in economic, legislative, social and cultural matters, including research. But the council, whose main meeting is an annual week-long session, can only make recommendations. By contrast, unanimous decision made by the Nordic Council of Ministers are binding on the governments, although in some cases subject to parliamentary approval. Meetings of the council are attended either by the ministers for Nordic cooperation or by the ministers responsible for a particular sector, such as culture and education.

Cultural cooperation within the region

is based on the 1971 Nordic Cultural Agreement which includes the areas of education and research. In education there is still no general Nordic recognition of qualifications although many of the courses and qualifications offered by arts and science faculties in higher education are acceptable in any Nordic country.

Cooperation in basic and applied research is enshrined in about 20 joint Nordic research establishments. Best known among those devoted to science are the Nordic Institute for Theoretical Atomic Physics in Copenhagen and the Nordic Volcanological Institute in Reykjavik. Funds for all joint ventures come from the Nordic Council of Ministers. A Research Policy Council, created in 1983, oversees developments and initiates new forms of cooperation. Nordforsk, the Nordic Council for Applied and Technical Research, awards substantial grants.

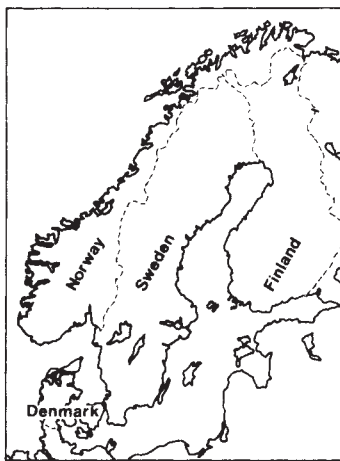
The inability of any of the individual Nordic countries to afford the heavy costs of some areas of research can be a prime reason for cooperation. One example is the proposed European Synchrotron Radiation Facility for which a Nordic consortium was formed to raise the price of buying into the project. For like it or not, no country of just a few million inhabitants, even such an exceptionally high spender on research and development in relationship to gross domestic product as Sweden, can mount an internationally competitive programme of research in more than a limited number of areas.

With 22 million inhabitants between them, the major Nordic countries could hope to compete in a larger number of areas were they fully to integrate their research programmes. But they do not. A traveller in the countries is likely to find lip service more prevalent than genuine enthusiasm for the idea of full-scale cooperation. This is as it should be. Care must be taken not to waste precious resources on the machinery of cooperation or on cooperative projects that are thought to be neither necessary nor desirable by those who are supposed to benefit from them. The partnerships that best suit research cannot be prescribed. They should be allowed to sprout and wither where and when is best for science, not for political ideals. Even if Nordic cooperation had to be constrained to allow more collaboration between individual sovereign states and countries elsewhere, the region would be likely to benefit just as much in the end.

Geography, acknowledgements and currencies

For practical purposes, as here, a map of the Nordic countries can only include the major countries (and the Åland Islands which lie west of the southern tip of Finland) because Iceland, Greenland and the Faeroes are inconveniently placed. That is little excuse, however, for not visiting them — particularly Iceland — before writing a survey of Nordic science. But it was not to be. As it was, Steven Dickman visited Denmark and would like to thank Peder Olesen Larsen, Mogens Dahl, Ove Nathan, Lauritz Holm-Nielsen and Bernard Jones; Peter Coles travelled to Finland, and is particularly grateful to Anne Ourila and Elisabeth Helander; Phillipa Lloyd thanks Richard Wright, Frank McGinley and Bjorg Svenggaard for their help in Norway; and Peter Newmark, who visited Sweden, is grateful for the help of Henry Danielsson and Sune Bergström.

The values of the Nordic currencies are



as follows: Danish kroner: £1 = DKr11.5; Finnish markka: £1 = FIM7.3; Norwegian krone: £1 = NOK11.4; Swedish krona: £1 = SEK10.8. □

Organization

Looking to science for a solution

THE high quality for which Danish products are known — be they sofas, ships or synthetic enzymes — is also reflected in Denmark's science. University administrators are quick to point out that the country has won more than its 'share' of Nobel prizes (eight all told), remarkable for a country with a population of only five million.

Denmark's success is all the more noteworthy for its meagre research and

Sciences, Medical Sciences, Technical Sciences, Agriculture and Veterinary Sciences, Social Sciences, and Humanities and by some *ad hoc* mission-orientated committees. The role of the six research councils should not be underestimated. They provide critical support for key programmes that might otherwise go begging. The decisions of the research councils are final, and cannot be questioned even by the Minister of Education himself. The

research councils will advise the government on the distribution of funds to the biotechnology programme and also a new materials sciences programme, when it begins.

Though the leaders of the research councils sometimes complain that insufficient university funding forces them to pro-

vide "band-aids" for some projects, these same leaders are blessed with flexibility almost unheard of within the universities. It is the bane of researchers in Danish universities that the amount of money given to a faculty is determined by teaching load. Exceptions for certain institutions — the Dansk Rumforsknings Institut (DRI), the Danish Space Research Institute, administered directly by the Ministry of Education, or the Niels Bohr Institute, a part of Copenhagen University — have allowed them to thrive, while in many other areas institutions have sunk danger-

ously close to mediocrity.

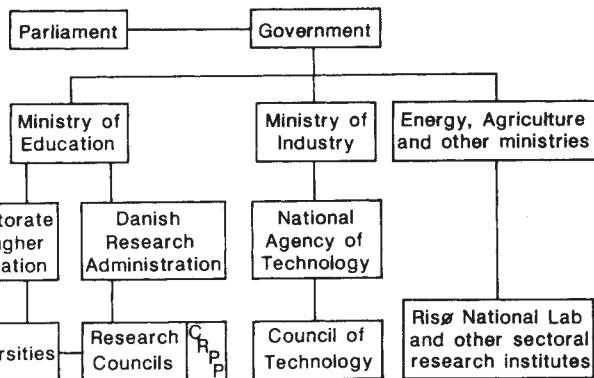
A small amount of the research carried out by private enterprise takes place in the twenty independent non-profit institutes set up in post-war years by the Akademiet for de tekniske Videnskaber (ATV) or Danish Academy of Technical Sciences. ATV was founded in 1937 to serve the needs of private industry. Only 20 per cent of the Dkr 350 million budget for the ATV Institutes (which include a bio-tech-

Distribution of 1987 R&D money

Organization	million Dkr
Research councils	200
Ministries of Education and Industry special programmes	750
Nordic and European participation	200
Universities	1,600
Medical science	500
Other public sector	200
Business enterprise	4,250
Non-profit	50
Total	7,750

nical institute, a maritime institute, and the international agency for ¹⁴C) comes from the Ministry of Industry; the bulk comes from outside contracts.

The rest of Denmark's private research is carried out by companies such as Novo Industries and the construction and high-technology firm Haldor Topsoe. Smaller firms, which predominate in Denmark, do hardly any research. The government is contemplating changes in the tax law to encourage more private R&D, but critics charge that this would only help twenty companies. Some suggest giving the tax breaks to the shareholders in smaller companies that do research as a more effective way to broaden the industrial R&D base. □



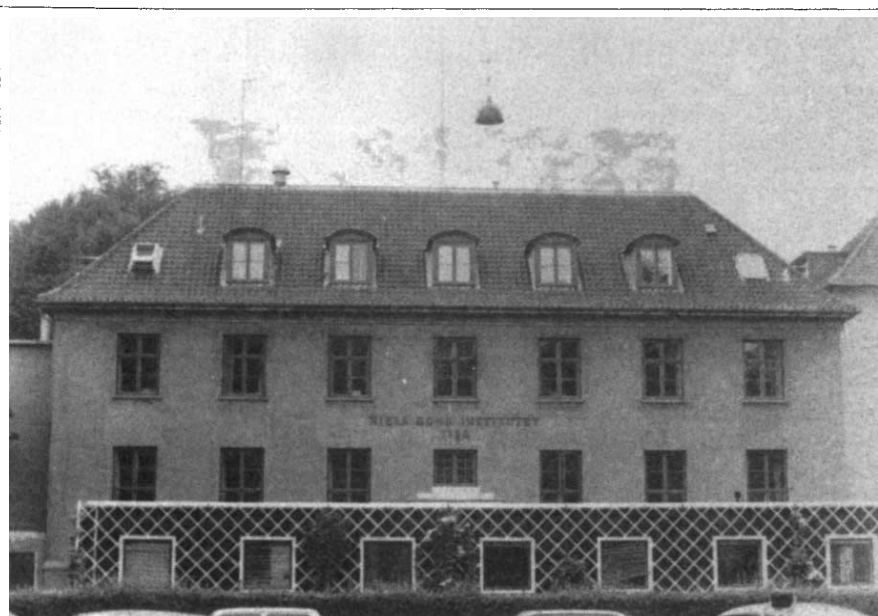
development expenditure, which at 1.1 per cent of gross domestic product (GDP), is less than half of the R&D investment in West Germany, Great Britain, and Sweden.

Part of the reason for this is that Denmark's industrial base is tiny, and what industry there is depends almost exclusively on imported technology (Novo Industries and Bang and Olufsen, the stereo manufacturer, are notable exceptions). As one senior science administrator puts it, "we Danes have always been known as good merchants, and that's what we still are. But perhaps we are good enough".

The Danish balance of payments is in a ruinous state; Denmark has the highest foreign debt per capita of any country in the world, as well as the highest taxes. Cutting costs at home is politically impossible — a rise in unemployment would surely bring down the fragile minority government.

So the politicians are looking to science for the answer, channelling much of their spending directly into the universities, (see table) and only a small proportion through the research councils. Some other government programmes — biotechnology is the current example, with an annual budget of Dkr 125 million a year for the next four years — are funded directly through the Ministry of Education. Also, each ministry has its own sectoral institute(s) for basic and applied research.

Provisions for key programmes are administered by the Council for Research Policy and Planning and the Council of Technology, the six councils — Natural



The cramped quarters of the Niels Bohr Institute, near the centre of Copenhagen. Founded in the 1920s, the institute is now looking for a lifeline to keep it going as explained on page 329.

Space science

Packaging irresistible instruments

SPACE science is one area where Denmark has obtained a firm grasp on its niche. "Our philosophy", says Niels Lund of the Danish Space Research Institute, DRI, "is to develop instruments for space science to the point where they're irresistible to those launching satellites". The most prolific launcher of the day — the Soviet Union — has found that Danish offer alluring in two cases of late: an all-sky monitor for hard X rays, due to be launched in November 1988, and a sensitive 8-metre X-ray telescope scheduled for launch in 1992.

Founded in its present form in 1966, the year Denmark joined the European Space Agency (ESA), DRI grew out of a programme of ionospheric research at the DTH (Danish Technical University). Crucial to its development was the insistence of its first director, Bernard Peters, that DRI should have technicians and engineers on the permanent staff so that it could produce its own space hardware. That way, DRI's director is free to make commitments to long-term international programmes. Without this freedom, "we wouldn't be on the [1992] Soviet satellite," says the current director, Herb Schnopper.

DRI is unusual in Denmark in being an institute commissioned to do research full-time, but with academic standards just as high as those in the universities. Schnopper compares it to the Smithsonian Astrophysical Observatory or the Woods Hole Marine Biological Laboratory in the United States. There are very few students at DRI, however, primarily because there is no funding for them, and this may eventually cause problems for the institute with its overseer, the Minister of Education.

The all-sky monitor, called WATCH (wide-angle telescope for cosmic hard X rays), was originally due to be launched on an ESA satellite on the US space shuttle in early 1988. The grounding of the shuttle pushed the scheduled launch back to 1991 at the earliest, so when the opportunity arose to launch WATCH with the French satellite GRANAT on a Soviet rocket in 1988, Lund was overjoyed.

There will be four WATCH instruments, along with six other devices from France and the Soviet Union, on the GRANAT mission. True to its name, WATCH will observe the whole sky for bursts of hard X rays, localize the bursts to within a fraction of a degree, and alert the on-board X-ray telescopes. WATCH will also be trained on known bright X-ray sources and will monitor their flux continuously — the project's "bread and butter", according to Schnopper.

Meanwhile, the Soviets are busy designing a spacecraft with a new type of

unfolding mechanism to accommodate DRI's 8-metre telescope, known as Spectrum X-Gamma. One reason that the DRI proposal succeeded with the Soviet space research institute IKI is that the mission is a balanced collaboration. The Soviets "don't just want to be the bus driver", says Schnopper.

Spectrum X-Gamma will operate over a broad energy range (from 100 eV to 2 keV) for a large number of different classes of sources. In particular, it is meant to examine the spectra of very distant active galactic nuclei and clusters of galaxies. It will be the largest X-ray astronomy mission to date when it is launched in 1992.

Despite its resounding success in attracting international collaborators, DRI seems unlikely to be given a larger slice of

the government's research pie. In fact, if a new government proposal is adopted, DRI will be forced to compete with other sectoral research institutes (those non-university institutions which are under the jurisdiction of the various ministries) for up to one-third of its budget. Although DRI would certainly hold its own in such a competition, Schnopper fears for his autonomy in signing up for more long-term projects.

Peder Olesen Larsen, director of the Danish Forskningssekretariatet (Danish Research Administration) and second only to the Minister of Education in Danish science administration, acknowledges that "success breeds success" in Danish astrophysics, and, all other things being equal, he recognizes that DRI would have a tendency to draw in ever-increasing funds based on its merits. But, he says, "we cannot allow it to grow too large if it doesn't have a closer connection to Danish production". □

Research Academy

Doctorates on the double

THE Danish government has become convinced that the key to its future is a guaranteed supply of brain power. So, in a forward-looking move, it last year created the Forskerakademiet (Research Academy) in Århus. The short-term goal of this experimental institution is to increase the number of doctoral degrees granted in Denmark from 180 per year to 360 or more by 1990.

There were several other goals in mind, according to the Academy's dynamic director, Lauritz Holm-Nielsen, who is a former Natural Sciences Research Council chairman. The idea was not only to fund more graduate students, but also to get them through the system faster (ideally in three years). The increased supply would in turn encourage sectoral research institutes to hire only people with doctorates. Also, the Academy is funding graduate students who do theses at the sectoral institutes themselves and in private industry, in order to bring these organizations closer to the academic world. And finally, it is trying to bring more coherence to a very uneven system of graduate education in the universities.

At first, the Academy evoked a typical Danish response — suspicion. "The universities were afraid that part of their competence was being taken away", says Holm-Nielsen, "and that the Academy would become some sort of super-university". Once the initial distrust was overcome, he adds, the universities all accepted the Academy and even welcomed it.

They discovered that Academy fellowships were flexible and could be granted in whatever faculty they were needed. Previously, graduate education was almost

exclusively supported by university budgets. This money was tied to the individual faculties and left the universities with scant manoeuvrability.

Sectoral institutes, too, have responded positively to the Academy. They are beginning to seek applicants with doctorates to fill their openings, which, says Ove Nathan, rector of Copenhagen University and a member of the Academy's



On trial: Lauritz Holm-Nielsen has five years to make a success of the Research Academy.

steering committee, will raise their standards and "those that refuse will be earmarked as impossible people".

Remarkably enough, the Academy is managing to accomplish all this on a relatively small budget — increasing from DKr 14 million in 1987 to DKr 150 million in 1992, when its five-year trial period ends. The low price-tag should help the Academy succeed, and success may make another government goal — to double the Danish investment in R&D in relation to GDP over the next ten years — a bit less unrealistic. □

Copenhagen University

Too much power to the people

RECTOR Ove Nathan of the University of Copenhagen is quick to show visitors a pitch-black cannonball hanging from the ceiling outside his office. The missile is a souvenir of the British bombardment of 1807 and the ensuing fire, which destroyed most of the university as well as much of the rest of Copenhagen. The onslaught was the Danes' punishment for siding with Napoleon.

That the university would be a target for

The other half is shared between faculty and the technical staff plus administration. Partly as a result of this development, politicians over the years have usurped as much authority as they could from the universities. When they want to initiate a programme, says Mogens Dahl of the Danish Research Administration, "they just create a new cigar box".

Under Copenhagen's democratic scheme, it is very hard to agree upon cut-

ting personnel. Consequently, when hard times descended, says Dahl, "they didn't cut people, they just took away their pencils". Reduced operating budgets have made it difficult to maintain good research.

Nathan, like other rectors, now plans to "use the present law to its very limits in order to steer toward more research-oriented universities and more continuity in

decisions". To this end, Nathan wants to increase the rector's power from "essentially zero" by making some money available for strategic research planning and



A quiet courtyard in the spread-out campus of Copenhagen University.

the cannons is fitting considering its central role in Danish intellectual society. Not only is Copenhagen University the country's largest educational institution, it is also the oldest, dating from 1479. Since then it has remained in the traditional continental mode, with five faculties, theology prominent among them. The other faculties are social sciences (which includes law and economics), humanities, medicine and natural sciences. Expansion has pushed most of the faculties away from the centre of the city.

Lately, another kind of battle has been simmering at the university. It is a tribute to Nathan that so few shots have been fired in the struggle over the allocation of limited government research funds. Says Lauritz Holm-Nielsen, director of the Research Academy (see previous page), "Nathan has done a lot in holding the diverse structures within the university together".

These "diverse structures", congenially called 'cigar boxes' by research administrators, make Copenhagen University an exceedingly difficult beast to manage. Not only is the structure atomized but administrators have very little power to change the system due to the legacy of the student movement in the late 1960s.

Student protests in Denmark were remarkably successful and resulted in a 1973 university reform roundly condemned for distributing power too broadly. According to the law, students have fifty per cent of the say in university policy decisions.

Århus University

Strength from competition

ALL Danish universities operate under the same democratic university law, but some are more democratic than others. At Århus University democracy does not get in the way of doing good research. In physics, mathematics and molecular biology, the faculties at Århus are said to be the best in Denmark, no small feat considering the competition in Copenhagen.

The healthy rivalry dates back to 1928, when Århus University was founded. It was the first Danish university outside Copenhagen and was private until as recently as 1970. Its 13,000 students are divided among five faculties of which natural sciences has 2,500.

What accounts for the relative lack of bureaucracy at Århus University besides its being far from the capital? Says Lauritz Holm-Nielsen, former dean of the faculty of sciences and currently director of the Danish Research Academy, "Democracy doesn't mean that no one takes responsibility; it just prescribes a system for electing people to take responsibility". Deans are stronger in Århus than in Copenhagen, he continues, and do not live in fear of being pushed out in the next elec-

What's in a title?

THE Danish Licentiat, granted for a thesis based on three years research following completion of the six-year Kandidatgrad, a Danish master's degree, is the equivalent of a PhD. But the difference in title causes difficulties for Danish students when they try to study abroad.

Since foreign study is such an important part of the Danish education, Copenhagen University's rector, Ove Nathan, and others are trying to rename the Licentiat, while creating uniformly high standards at all Danish universities to conform with a "good US PhD".

The traditional Danish Doktorgrad will not be affected. Getting a Doktorgrad involves five to ten years of independent work beyond the Licentiat. It is similar to the German Habilitation or the French Doctorat d'état, which has been abolished. The Doktorgrad confers no tangible benefits, says Nathan, with one exception. A Doktor may not be denied the right to give a public lecture at a university. This right is only exercised "when people get really mad at each other". □

development. This would give him the opportunity "to secure the marginal additions [to the research budget] that we all know are so important". The rectors of all the Danish universities will meet with Education Minister Bertel Haarder on 26 November to present their recommendations. □

tion. When he was Dean in the 1970s, Holm-Nielsen made sure that operating costs for research groups were not reduced, even when it meant not hiring the full complement of staff.

Holm-Nielsen also credits former dean Svend Bundgaard both for keeping more central authority in the hands of the Århus deans and for encouraging foreign study. "Århus has a tradition", he explains, "that you can't get a faculty position unless you've been abroad".

Århus physicists will benefit from a dual-purpose synchrotron now being constructed. It will be unique in storing both heavy ions (from protons up to actinides) and electrons, albeit not simultaneously. The physics faculty is planning to use the facility, which will operate in the MeV range, for six months at a time in each mode. The synchrotron may provide opportunities for industry, for example in micro lithography, but the first non-physicist collaborators are likely to be chemists and even biologists. There is discussion of building an X-ray microscope similar to that in Daresbury, England or at DESY in Hamburg. □

Bohr Institute

Niels Bohr's long legacy

WERE Niels Bohr still alive — he died in 1962 — he would probably find some way to rescue the Copenhagen-based institute that now bears his name from its current doldrums.

Bohr founded the institute in 1921, the year before he won a Nobel prize in physics (a feat that was repeated by his son, Aage, in 1975). But his influence on Danish physics did not stop there. He was also instrumental in guiding the Forskningscenter Risø on a path of basic energy research instead of pure nuclear physics, a move that allowed it to survive when Denmark later decided not to build any nuclear power plants.

As if that is not enough, Bohr's collaboration with the Russian physicist Lev Landau established the basis for a wide-ranging scientific exchange between Denmark and the Soviet Union. Even during the difficult days in the early 1980s, some 20 Soviet physicists a year were visiting Denmark for up to several months. Next year, 25 Soviet researchers are due in Copenhagen to celebrate the eighteenth anniversary of Landau's birth.

Bohr can also be credited with the

special status that the institute has enjoyed with regard to government funding, as well as with the tendency of Danish industry to start to pick up the slack as government funds start to falter. Danish physics has responded by achieving significantly more scientific citations per physics paper than Great Britain, West Germany and France, according to a British Royal Society study.

But, according to director Knud Hansen, the Bohr Institute has come upon hard times in recent years. Despite quite a few permanent positions for researchers not required to teach, Hansen says that the university "budget model" has "no room left" for research institutions like his.

The addition of five more five-year research professorships is only a stopgap, according to Hansen. "It's like a man drowning in the middle of the Atlantic clinging to a piece of driftwood". If something does not float by soon, Hansen says, the high standards of the institute are at risk. Will Bohr's posthumous influence, which is still strongly felt in the corridors of power, be enough? □

Greenland

Did Erik the Red see green?

How did Greenland, the icy island that is still controlled by Denmark despite the recent introduction of home rule, come by its name? Willi Dansgaard and his colleagues at the Geofysisk Institut (Geophysical Isotope Laboratory) of the University of Copenhagen may have the answer.

Through the study of oxygen isotopes present in cores drilled from the Greenland ice sheet, Dansgaard and his colleagues have developed a theory relating the presence of ^{18}O isotopes to climate. They suggest that Erik the Red, the Norse explorer who discovered Greenland in 982, arrived at the end of a warm period lasting nearly 100 years that left the island green and

which is longer than any that has occurred since. By contrast, the Greenland Saga claims that Erik called the new land Greenland only to persuade people to follow him there. Erik's settlement in southern Greenland disappeared mysteriously in the mid-1400s, perhaps because of a prolonged cold spell or raids by pirates.

Denmark has long sent scientists to Greenland to study the ionosphere and the Earth's magnetic field, taking advantage of the clean air and the proximity of the north magnetic pole. Moreover, polar regions are where climatic changes tend first to make themselves felt and ice cores retain the record. The Greenland Ice

Nordic collaboration

LOCATED in one wing of the Niels Bohr Institute, NORDITA, the Nordic Institute for Theoretical Physics, is "the most institutionalized scientific collaboration" among the Scandinavian countries, according to its director, Allan Mackintosh. Its budget of DKr15 million for 1987 is provided on a GDP basis by the five Nordic countries (including Iceland). And its undoubted success is attributed in large part to Niels Bohr, who helped to found NORDITA in the late 1950s.

The institute's official purpose is "to further collaboration between the Nordic countries within basic theoretical physics". There are 30 to 40 researchers there at any given time, including 6 full professors, 4 assistant professors and up to 16 Nordic fellows who are resident for two years before returning to their home countries. The institute acts as a focal point for theoretical physics in the Nordic region and as a draw to distinguished visitors from elsewhere. Part of its budget is used to allow visitors to spend a period of time in institutes in the member states.

The institute is funded by the Nordic Council of Ministers and is run by a board composed of physicists from each of the member countries. The board and the director keep a watchful eye on the balance of the staff in terms of their country of origin.

Mackintosh realizes just how lucky he is to be in an institution with a stable budget. NORDITA has even been able to get some sorely needed increases from the member states — for computing, for example. By comparison, the Bohr Institute had to rely on a private donation two years ago to update its Univac computer. □

Sheet Program, a joint Danish-Swiss-US project, sent groups led by Dansgaard, Hans Oeschger of Switzerland, and C.C. Langway of the United States to a site in southeast Greenland for three successive summers in the mid-1970s. There they drilled back in time tens of thousands of years, testing the core samples for carbon dioxide content as well as for cosmic dust and oxygen isotopes.

Dansgaard and his international counterparts now have an even more ambitious programme in mind. They would like to set up another drill at "SUMMIT", the highest (and coldest) point in Greenland, and accomplish two tasks. First would be to examine in detail the dust blown in from other land masses that has collected there over the past 1,000 years and which would help in dating climatic changes. Second would be to go back 500,000 years in time — more than was possible at the first site. Dansgaard hopes that the project will be blessed by the European Science Foundation by the end of 1987. This would help with the fund-raising effort. It is hoped that drilling will begin in summer 1989. □



Removing an ice-core sample that dates back approximately 50,000 years to the glacial period.

Technical university

Fears of an engineering gap

THE Danish Technical University (DTH) dates back to 1829, when it was established by the illustrious Hans Christian Ørsted, who first demonstrated the connection between electricity and magnetism. Shunted from venue to venue over the years, DTH finally found a permanent home during the fat years of the 1960s, when it was established on the grounds of a former airfield in Lyngby, 15 kilometres from the centre of Copenhagen. The move was completed in 1973, and the end result was a tripling in size.

DTH admits 1,000 students a year, granting both *Civilingeniør* (Master of Science in Engineering) and *Licentiat* (Doctor of Engineering) degrees. About 80 per cent of the students are in the former programme.

Research is carried out on an internationally recognized level in areas such as microelectronics, computer-aided design and applied physics. A permanent research staff of about 500 is augmented by 300 short-term staff and 150 international visitors a year. Special priority areas have been set up to try to keep Denmark's research in step with the inter-

national competition. Notable among these is MIDIT, a programme in modelling, non-linear dynamics and irreversible thermodynamics.

DTH faces considerable difficulties in the years to come. Heading the list is the expected shortfall of engineering students highlighted in a recent Organization for Economic Co-operation and Development (OECD) report on Denmark's national science policy. The OECD warned that despite an absolute increase in the number of engineering students (the number of civil engineering graduates nearly doubled from 1977 to 1986), universities will be hard pressed to train as many young engineers as Denmark needs.

In addition, DTH is plagued with that ailment familiar to most engineering schools of not being able to attract enough young faculty members. Salaries in Danish universities "can't compete with those in private industry", says DTH rector Hans Peter Jensen. The company is far from being a ghost town, but the implication is that the 1990s will bring not enough students, and not enough teachers to teach them. □

Carlsberg Laboratory

Not crying in their beer

SOME of Denmark's happiest scientists work for a brewery. But the basic researchers at the Carlsberg Laboratory are not there just for the beer. With a budget (DKr 60 million) as large as that of the Natural Sciences Research Council, the Carlsberg Laboratory is one of the most highly respected institutions in Denmark.

Founded in 1875, it was established to develop "as fully scientific a basis as possible for the operations of malting, brewing and fermentation". The laboratory, which carries out basic and applied research, was augmented in 1976 by the Carlsberg Research Center, which does only applied research. Both are owned by United Breweries, which produces the Carlsberg and Tuborg brands of beer. The breweries' majority stockholder is the Carlsberg Foundation, which also gives grants to university researchers and supports artistic endeavour in Denmark.

The laboratory's two departments, chemistry and physiology, have been directed by a series of world-class researchers, starting with E.C. Hansen, head of physiology from 1887-1909, who developed the first pure culture of yeast, a boon to brewers and biologists alike. S.P.L. Sørensen, head of chemistry from 1901 until 1938, was famous for the buffer system that served for years as the standard for the definition of pH.

The current head of physiology, Diter von Wettstein, continues the tradition of excellence. Von Wettstein, a fourth generation biologist, has interests in plant breeding as well as molecular biology. The group of researchers he has assembled includes Morten Kielland-Brandt, whose group works on changing the efficiency of certain enzymes in brewer's yeast using the techniques of molecular biology. Should the effort succeed, the average



Diter von Wettstein, head of physiology at the Carlsberg is a "fourth-generation biologist".

brewing time of beer might be reduced from several days to several hours.

The chemistry department is using site-directed mutagenesis to change the specificity of the yeast enzyme carboxypeptidase Y, which is useful for peptide synthesis. If the goal of producing a variety of specificities and having the enzyme secreted from the cells instead of being stored in a vacuole is achieved it

Århus Science Park

Look for industry on the windswept Jutland peninsula, and all you are likely to find is a Lego factory pressing toys out of plastic. Although the chemical and electronics industries are making inroads on Jutland's pastoral setting, much of its economy is still agricultural. But now a small group of people in Århus are sowing the seeds for the kind of high-tech industry that makes government ministers salivate. With enthusiastic backing from Århus University, they have started Århus Science Park, which is still not much bigger than the tiny saplings planted neatly in its courtyard.

The philosophy of the park is conservative. Instead of trying to be a publicly financed 'motor' for technology development in the area, it is simply providing space for technology development "with the objective of creating research-based production". The aims fit in with the desires of the Danish government to link basic research with industry in such a way as to increase productivity.

Local industry showed enthusiasm for the project from the start. Banks, companies, and the Århus University research foundation contributed to an initial investment of DKr 9 million, which was used to lease the land and erect the first buildings. The number of local 'member firms' has risen from 21 in 1985 to 125 in mid-1987. Tenants include a branch of the Danish water quality institute, a cancer virology laboratory supported by the Danish Cancer Society, and an engineering firm trying to find ways to recycle the waste products from fish processing.

Science Park academic administrator Jørgen Anderson (one of a total of four employees) explains that the general pattern of support within the EEC for innovation parks may catch on in time. But in Denmark, it is necessary to start smaller, because "most firms don't have a tradition of having scientific people in their labour force". Jutland, he explains, is even more conservative than the rest of Denmark in this respect. The thought of spinning off projects from Århus University, a few blocks away, is "still a dream". □

could be of major significance for the biotechnology industry.

But it all comes back to beer. As von Wettstein puts it, "Of course we want to help improve the quality of the beer — the more beer that's sold, the more money we get to do research". In general the brewers do not pay too much attention. At one point, however, the Carlsberg Research Laboratory discovered that the taste difference between Carlsberg and Tuborg is accounted for by their 3-methylbutylacetate content. That was, says von Wettstein, "the first time we made an impression on the brewers". □

Policy and funding

Not yet back to basics

THE present apparatus for science policy development and funding in Finland was established in 1969 and added to in 1983 when a parallel infrastructure for technology research was set up. Having realized relatively late the need for an organized science and technology research policy, Finland is working hard to make up for lost time.

Over 75 per cent of research in Finland is financed by two ministries. The Ministry of Education funds universities and institutes of higher education directly and also funds basic research indirectly through the Academy of Finland. The present Academy, restructured in 1969, consists of seven research councils and a Central Board of Research Councils. The Academy has become the principal organ for development and implementation of basic research. This year, the Ministry of Education will spend FIM 1,052 million on research, 23 per cent of which will go to the Academy of Finland.

Most of the remaining government expenditure on research and development (almost 40 per cent of the total state research budget) is routed through the Ministry of Trade and Industry. In 1983, the Technology Development Centre (TEKES) was set up by Act of Parliament to plan and allocate state funding for technology research. It coordinates links between universities, institutes and industry, with an overall aim of promoting the international competitiveness of Finnish industry, largely through grants and loans for goal-oriented research. This year, TEKES's budget increased by 13 per cent to FIM 425.5 million. The Ministry of Trade and Industry and TEKES also provide about 25 per cent of the budget for the Technical Research Centre of Finland (VTT), the largest technical research institute in the Nordic Countries.

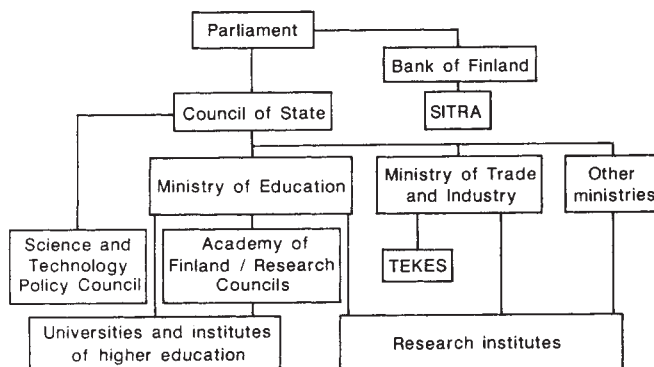
The Finnish National Fund for Research and Development (SITRA) is a third source of research funding, set up in 1967 to commemorate 50 years of Finnish independence. It mainly finances development of industrial production in high risk areas and, in 1984, gave out awards totalling FIM 68 million.

As Finland is a neutral country it spends little on defence research. The Ministry of Defence research and development budget for 1987 is FIM 47.5 million. Research spending by a combination of other ministries accounts for about

21 per cent of the total state research budget for 1987.

The practice of basic research funding follows a common pattern. The research councils of the Academy of Finland receive applications from faculty members of universities and research institutes, and then award project grants, on the basis of merit, for fixed periods. In addition, the Academy supports some 600 research posts at various levels.

Development of science policy and planning started in Finland in 1963, when the Science Policy Council was set up. Renamed the Science and Technology



Policy Council in 1987, it assists the Council of State (the government's highest executive body) and its ministries in issues related to science and technology. The Council is chaired by the Prime Minister and its membership comprises the ministers most concerned with research and representatives from the Academy of Finland, TEKES, research, higher education and industry.

In its current programme, running until 1990, the Science and Technology Policy Council is concerned with four main trends: ensuring constant growth of research and development, achieving a better balance between growth and development, researcher training, and internationalization of Finnish research. It is up to the Academy of Finland and TEKES to translate these into specific initiatives.

Elisabeth Helander, research director of the Academy of Finland, explained the need to monitor growth in research: "Finland spent only 1.2 per cent of gross domestic product on R&D in 1985. This year, it will spend 1.7 per cent and the goal set by the previous government is 2 per cent by 1990, giving Finland the highest growth rate in research spending of any OECD small country". The proportion of the overall national research budget allocated for basic research has, however, shown a net decline over the past decade. The growth therefore reflects an increase in state investment in applied research.

The Finn red line

"POLAR bears don't walk the streets of Helsinki, we are not communists and nobody speaks Russian". These words may be found in tourist guides distributed to Finnish hotels, but also spring readily to the lips of scientists and administrators faced with a foreign journalist who wants to write about their country.

Finns are sensitive about their border with the Soviet Union, pointing to the lack of affinity between the two countries. From the twelfth century until 1809, Finland was a part of Sweden. Even today, 300,000 Finns (6 per cent) have Swedish as their mother tongue and the country is officially bilingual. There is even a Swedish-language university (Åbo Akademi) in Turku. Government from Russia lasted only a century. In 1809 Finland was made an autonomous Grand Duchy, ruled by the Tsar of Russia, until attaining its independence as a republic in 1917.

After the Second World War, Finland lost part of its territory (Estonia and Karelia) to the USSR but prides itself on having never been occupied. With war damages to pay to the United States until the early 1950s, Finland developed a neutral foreign policy that persists today.

Finland's modern front door and facade may face west, but the back door opens onto 8.5 million square miles of Soviet Union. If Finland is the Western department store most accessible to the Soviet Union, the USSR offers energy resources, world-class theoreticians, especially in mathematics and physics, and a ready market for Finnish high-tech exports. □

There are attempts to redress the balance between growth and development and to redirect more money to basic research, especially in the universities. "The second ten-year Development Law, passed by Parliament last winter", says Esko-Olavi Sepälä, the Science and Technology Policy Council's chief planning officer, "was accompanied by a government decision to give universities a real budget increase of 15 per cent per year for the next four years". This new money is intended for research and also to improve training, by increasing the number of places and decreasing the time it takes to complete a PhD (see next page).

One of the major aims of the current programme is to increase the 'internationalization' of Finnish science and technology. The Academy of Finland plays a significant role in this effort, both by providing funds for researchers at all levels to travel abroad and by its membership of European projects. Similarly, TEKES has increased its role in promoting international contacts, for example through the European Space Agency, of which Finland became an associate member this year. □

Internationalization

Lig big science to the rescue

'INTERNATIONALIZATION' is a watch-word of the Finnish strategy for tackling the ubiquitous problem that small countries have of carrying out research on a shoestring but at the leading edge of a number of disciplines. The word is used to describe both the goal and the means to attain it. Some countries are more 'international' than others, though, and some connections are more coveted.

For Finns, internationalization means the exchange of scientists and the sharing of 'big science' between countries. It also means developing prominence in areas that are manageable on a small budget. Finns are proud of their successes in this last area — 'little big science', as it has been called. As Markku Mannerkoski, director-general of the Technical Research Centre of Finland (VTT), puts it, "we can't make the rockets, but we can make the instruments".

In basic science, collaboration between research groups is often necessary for expensive research, and, according to Jonathon Knowles, research professor at the Biotechnical Laboratory of VTT, "here you know everybody. It can be much more parochial in larger laboratories. Here you are forced to collaborate". Just that situation contributed to Finland being the country where inter-

feron was first purified (see below).

This advantage also applies to horizontal communication between different research administrations, like the Academy of Finland and TEKES, or between university and industry. "Isolation of organizations is a big country problem", says Juhani Kuusi, director-general of TEKES.

When Finnish scientists talk of establishing international contacts, they usually mean contacts with the United States and Europe. "Cooperation between Nordic countries is very important, but it is so natural and common that we tend to forget it. We consider this as domestic", says Markku Mannerkoski. Through the Nordic Consortium, Finland hopes to be able to participate in the European Synchrotron radiation source at Grenoble.

Finland is also contributing, on a small scale, to a number of other European projects, such as the European Space Agency, of which it became an associate member this year, as well as several Eureka projects, organized through TEKES.

Connections with the United States, especially in biomedical areas, are particularly strong. Because Finland repaid its entire Second World War debt to the USA by the early 1950s, American research foundations, such as the National Institutes of Health (NIH) and Fulbright readily gave postdoctoral fellowships to Finns — six each year in medical sciences.

Many of the most successful Finnish research groups were established by scientists who profited from these fellowships in the 1950s and 1960s. Helena Mäkelä, for example, now director of the bacteriology department of the National Public Health Institute in Helsinki and chairperson of the Medical Research Council of the Academy of Finland, introduced bacterial genetics to Finland after postdoctoral training in the United States.

Some senior scientists are able to maintain transatlantic collaboration with grants from the United States and some money from the Academy of Finland. Nearly 60 per cent of the FIM 12 million budget of the Public Health Institute currently comes from abroad, either from the World Health Organization or from NIH, for field trials on vaccines and for a study of vitamin A therapy for cancers.

For many, collaboration with the Soviet Union does not count as 'internationalization'. There is a feeling, justified or not, that Soviet scientists need Finland more than the reverse. It is a question of "ystävyyks ja yhteistyö" — friendship and cooperation, the basis of Finland's diplomatic relationship with the Soviet Union.

Professor Pekka Halonen, head of the

Small is beautiful

WORKING in a small country does have advantages, as a recent study of AIDS (acquired immune deficiency syndrome) in Finland is proving. A well-established national health service has encouraged public willingness to co-operate with doctors. For epidemiological studies, this is giving Finland the edge over some bigger countries.

In 1983 Sirkka-Liisa Valle and Annamari Ranki of the Helsinki University Central Hospital and Kai Krohn of the Institute of Biomedical Sciences at Tampere University started to build up a cohort of homosexual men and their partners, in order to look at T4 and T8 lymphocyte ratios. The cohort has now grown to almost 300 and in four years there have been almost no drop-outs.

"Our cohort is relatively small, but it is very stable", says Ranki. "In the United States it would be almost impossible to keep track of this kind of sample. The participants are also willing to help, so that it has been possible to perform spinal taps as well as taking samples of blood. This again would be very difficult in the United States." A tendency for the cohort to heed medical advice on precautions against AIDS infection, Ranki speculates, may have contributed to the finding that HIV (human immunodeficiency virus) antigens, or antibodies to recombinant proteins were seen 6 to 14 months before seroconversion in the 9 subjects who seroconverted.

An epidemiological study on AIDS has now started and blood samples are being routinely taken from outpatients at some hospital clinics. Patient participation is voluntary, but few have declined. □

Department of Virology at Turku University, explains that "Finland's expertise in molecular biology is particularly valued in the Soviet Union, where techniques may be unknown and materials unobtainable. We send tens of thousands of reagents to collaborating laboratories in the Soviet Union. We also often have anywhere between 3 and 5 visitors to the laboratory from the USSR".

In radio astronomy and nuclear physics, collaboration between Soviet and Finnish scientists is more balanced. By comparing observations from radio telescopes in Finland and the Soviet Union researchers at Helsinki University of Technology's Radio Laboratory have access to a highly sensitive observation system.

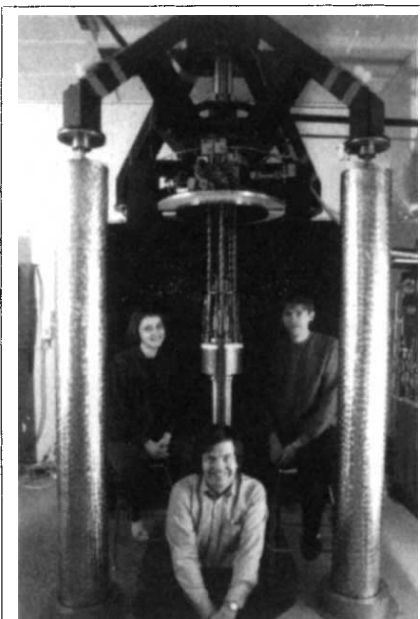
Finland will also contribute to the Soviet Phobos mission to Mars, although Antti Räisänen, of the Radio Laboratory, anticipates difficulties with COCOM regulations, particularly regarding the gallium arsenide 22 GHz receiver he is due to build since the only three suppliers of gallium arsenide are in the United States, France and Japan. □

The first interferon

It was because of a friendship between Kari Cantell of the National Public Health Institute and the chief professor of the Blood Transfusion Centre there that Cantell was able to get sufficient supplies of buffy coat separated out from blood units to achieve, in 1963, the first purifications of interferon.

"In our best years, we produced six billion units of natural interferon but not a single unit of blood was collected for this purpose. All of the buffy coat we supplied to Dr Cantell was produced by removing unwanted leukocytes from blood units", says G. Nyllylä of the institute's Blood Transfusion Centre. When the value of interferon was appreciated, the US NIH provided Cantell and his co-workers with funds for large scale purification.

Today, 'recombinant' interferon has taken over, but the Finnish natural product may have accidental properties that the recombinant product lacks. "Our product is rather impure", explains Nyllylä, "but it contains all or most of the subtypes of alpha interferon. From *in vitro* experiments we know that these have a synergistic effect. It remains to be seen from clinical trials whether this 'soup' of alpha interferons is more effective than the single type produced from recombinant DNA". □



The ROTA-II cryostat in the Low Temperature Laboratory at the Helsinki University of Technology; the apparatus is used for investigating vortices in rotating superfluid³He at temperatures between 0.5 and 2 mK. Project leader Peter Berglund in front. ROTA is an example of the international aspect of Finnish science — being a joint undertaking between the Academy of Finland and the USSR Academy of Sciences.

Researcher training

Two doctorates to a PhD?

THE average Finn is 34 years old when he defends his doctoral dissertation, compared with a mean age of 27 years in the United States and Great Britain. As this threatens to retard Finland's attempts to improve its international competitiveness, the Academy of Finland has started to take corrective measures.

The Finnish doctorate is much more than a PhD. Traditionally, both an MSc and the successful submission of a licentiate dissertation (equivalent to two research papers) are required before going on to study for a doctorate. "The threshold of getting a PhD is too high. It becomes like a final goal", feels Niilo Saranuumi, director of the Medical Engineering Laboratory at the Technical Research Centre of Finland (VTT).

Olli Lounasmaa, research professor at the Low-Temperature Laboratory of the Helsinki University of Technology thinks it is "ridiculous" for a PhD to take 8 years. "The real reason PhDs take so long is that students don't get enough supervision. You need both the carrot and the stick". Lounasmaa's group has produced 31 PhDs since it started in 1965 and he expects 9 more this year.

It is no longer mandatory to submit a

Universities

Regionalization policies in action

UNTIL 1917 Finland had only one university, which had been transferred in the nineteenth century from Turku, on the southwest coast, to Helsinki. Now there are nine universities, where most of the country's basic research is carried out, three technical universities and the modern Technical Research Centre of Finland (VTT) at Otaniemi, outside Helsinki, which is the largest technical research campus in the Nordic countries.

The expansion of the university system took place in the 1960s and 70s as part of a government regional development plan, in response to a powerful agro-political lobby. The aim, says Esko-Olavi Seppälä, chief planning officer of the Science and Technology Policy Council of Finland, was to use higher education as a means to stimulate rural areas. "The regional universities have been successful from this point of view", says Seppälä, "but in terms of science policy, they were too small to be effective. The Academy of Finland had to put in a lot of money in the 1980s, but we are now in a position to concentrate on

science policy, not on walls".

One of the most successful of the regional universities is at Oulu, on the southern edge of Lapland and founded in 1958. Oulu has also recently become the site for the Nordic countries' first science park, with some of Finland's most prominent technology companies, such as electronics firm, Nokia-Mobio Oy.

According to Seppälä, the absence of a regional university system in other Nordic countries, particularly Norway, is causing "real problems". But it may have disadvantages, too. Kai Simons, Finnish representative at the European Molecular Biology Laboratory at Heidelberg, West Germany, feels that the duplication of resources throughout a country the size of Finland must make it harder to develop world class research groups. "Finland has to make a decision on these universities", says Simons. "The best biomedical group outside Helsinki is at Oulu, but you can't have biological research all over the country. There are five medical schools, for a population of 4.9 million". □



VTT near Helsinki. The largest technology institute in the Nordic countries and a showcase of university-industry collaboration.

licentiate dissertation before starting doctoral work, and more graduate students, as in Lounasmaa's group, are being accepted at MSc level. But in fields such as molecular biology and virology it is not uncommon to find 'double doctors', with both an MD and a PhD, taken one after the other or in parallel. Some, like Kai Krohn, professor of pathology at the University of Tampere, believe this gives Finns an advantage compared with many of their European and American colleagues.

The Academy of Finland has started to tackle the problem of postgraduate training and offers a number of three-year research assistantships, since many of the university assistantships — intended for

postgraduates — are occupied by postdoctoral researchers unable to find suitable junior lectureships. To ease the situation, the Academy has created some senior research positions, but the shortage of early and mid-career faculty posts has forced some researchers abroad.

The long PhD training has also discouraged industrial R&D departments from employing scientists with doctorates. They are often considered too old and too specialized. Now there are worries that researchers in Finnish industry are under-qualified. In response, the Academy now offers about half (FIM 5,700 per month) of the salary of private sector researchers released to study for a PhD. □

Biotechnology

Into trees but not out of the wood

IN 1960, forestry and the wood, pulp and paper industry accounted for 69 per cent of Finland's exports. The proportion has since dropped to 36 per cent, largely because of a policy of diversification in the nation's technology, but with 65 per cent of the country covered by forest, it is not surprising that new technologies, like genetic engineering, have a bias towards traditional applications.

Most of the trees in Finland's forests are pine and spruce, but it is the long-fibre properties of South American eucalyptus, which cannot grow so far north, that are most in demand for contemporary paper-making. Birch is the only native species



Dr Veli Kauppinen with birch embryos produced from 'artificial seeds' at the VTT Biotechnical Laboratory.

that can compete with eucalyptus, but it has been difficult to cultivate on a large scale. Using biotechnology techniques, a research group at the Biotechnical Laboratory of the Technical Research Centre of Finland (VTT) thinks it has found a solution.

"The problem", says Veli Kauppinen, who is directing the research, "is that rodents eat up to 80 per cent of our birch seedlings in winter. It is the presence of secondary metabolites, which do not taste nice, that allows the other 20 per cent to survive".

The rodent-resistant variety cannot be multiplied by seed, since the trees lose their resistance, while vegetative techniques are too laborious for commercial applications. So Kauppinen has developed cloning techniques to make artificial seeds, which are then used to produce embryos in suspension. These can then be cultivated.

In the same laboratory, Jonathon Knowles is working in collaboration with the Finnish Pulp and Paper Research

Institute, a private corporation owned by the Finnish paper industries, to look at ways of producing new enzymes to replace chlorine chemicals in wood bleaching — part of a national drive to reduce pollution of Finland's lakes.

Knowles, an Englishman, was one of a group of scientists who introduced recombinant DNA techniques to Finland at the beginning of the 1980s, as part of a 3-year research project funded by SITRA, the Finnish National Fund for Research and Development. Housed at the Meilahti Institutes and the National Public Health Institute in Helsinki, the project brought together virologists, bacteriologists, and protein and nucleic acid chemists, and gave Finnish biotechnology the start it needed.

Following on from this project, a recombinant DNA laboratory was set up

in 1982 by the University of Helsinki, with funds from the Academy of Finland, the Ministry of Education, SITRA and Finnish industry. Many of the original researchers have now left to form their own research groups and, this year, Genesit Oy, an industry-sponsored group working within the university recombinant DNA laboratory on the development of host and vector systems in *Bacillus*, will become an autonomous commercial enterprise.

Nevertheless, a report published by the Academy of Finland this year says that an extra financial input of FIM 37 million per year, for the next five years, will be needed in order to bring Finnish biotechnology and molecular biology research up to the standard required to compete internationally. The Academy has targetted research training and new equipment as specific goals and hopes that different sectors of government, such as the Ministry for Agriculture and Forestry, will provide special funding for research groups. □

Energy

Hobson's choice on nuclear power

OVER the past decade Finland has become one of the top four nuclear nations, currently producing 34 per cent of its electricity and 16 per cent of all energy by nuclear power. Energy-intensive process industries, the cold climate and long, dark winters help make Finland's energy consumption per head one of the highest in the world, behind only the United States and Sweden. Yet it has no high-grade fossil fuel resources and only limited potential for hydropower. Although there are over 55,000 lakes in the country, they contain relatively little water and the terrain is flat.

As a result, energy, particularly in the form of oil, accounts for 16 per cent of the value of all imports with 76 per cent coming from the Soviet Union. Finland has tried to diversify its energy sources, but its options are limited. As in Sweden, peat is plentiful, but seasonal restrictions on harvesting and high transportation costs make it an impractical fuel on a large scale. Pollution of lakes and forests by coal-burning electricity generators make them increasingly unacceptable. A bilateral trade agreement with the Soviet Union, signed just after the Second World War, means that Finland cannot increase energy imports from its neighbour without increasing its exports, which is impossible so long as oil prices remain low.

Nuclear power therefore became a natural choice for Finland, especially after the oil crisis of the 1970s. There are currently four reactors in operation, producing a total of 2,300 MW. The state-owned Imatra Power Company operates two 440-MW pressurized light water reactors, are

situated at Loviisa, about 100 km east of Helsinki and were built with components supplied by a Soviet export organization, V/O Atomenergosexport, better known as 'Eastinghouse'.

The other two reactors are operated by the Industrial Power Company, a private consortium with the state as 50 per cent shareholder. These 660-MW Swedish-designed boiling water type reactors are situated on the south-west coast, at Olkiluoto.

Plans to build a fifth reactor were suspended by the present coalition government following public nervousness after the Chernobyl disaster, even though Finland was less affected than was at first thought. Finland still needs to find an alternative source of extra power to cope with a forecast growth in demand. During last winter's severe weather, the country found itself with only 0.4 MW spare capacity at peak times.

Unlike Sweden which is committed to life without nuclear power by the year 2010 (see page 342), Finland does not intend to shut existing reactors. When the incumbent government's mandate expires in 1991, plans for the fifth reactor may even be dusted off. Whatever happens, a major problem that is more than likely to face Finland's nuclear industry in the next decade will be the recruitment of sufficient nuclear engineers. Once considered a glamorous occupation, the nuclear industry is now finding it hard to fill vacancies, while nuclear engineering departments of Finland's technical universities are attracting fewer students. □

Organization

Pluralism and priorities

NORWAY takes a very pluralist approach to its research policy and the distribution of funds for research and development. Parliament (Storting) is the highest authority for science and technology policy but remains distanced from R&D problems. Various committees advise and coordinate the nation's policy and there is a sectoral policy towards research organization with individual ministries having decision-making power over the R&D activities within their field of responsibility.

The highest government agency is the Cabinet Research Board (RFU), which is chaired by the Minister of Cultural and Scientific Affairs and consists of the seven ministers with the greatest R&D responsibilities. RFU's main tasks are to consider questions of science policy, to define government priorities in areas of national interest, to prepare reports for the Storting, to promote cooperation between the Norwegian research councils and to determine the extent to which Norway should participate in international research programmes on the basis of a national science policy.

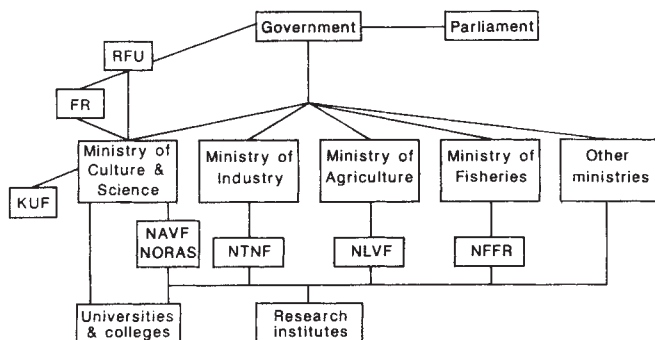
The Science Policy Council (FR) acts as an advisory body for the RFU, in research and providing information and opinions on which to base a national science policy. The committee of twelve is appointed by government and is mostly drawn from the scientific community on both the academic and industrial sides. For its first ten years FR was attached to the Prime Minister's office, but since 1975 it has been attached administratively to the Ministry of Cultural and Scientific Affairs. However, says Arnhild Hole, director general of the ministry's research division, "the government does not really know how best to use this committee, which does not play a strong enough part in forming science policy".

The most recent addition to the governmental machinery is the Research Contact Committee (KUF), which was set up in January 1987 to replace the Interdepartmental Research Committee. The new committee, consisting of senior civil servants and officials responsible for research in most ministries, promotes open discussion between ministries, research councils and research centres.

Support for research in Norway is channelled through all seventeen ministries, but primarily the ministries of Cul-

tural and Scientific Affairs, Industry, Agriculture, and Fisheries. Other research funds are distributed by the five research councils: NTNF, the Royal Norwegian Council for Science and Industrial Research; NAVF, the Norwegian Council for Science and the Humanities; NLVF, the Agricultural Research Council of Norway; NFFR, the Norwegian Fisheries Research Council; and NORAS, the Norwegian Research Council for Applied Social Science Research.

The largest of these, NTNF, is attached to the Ministry of Industry and is associated with sixteen research institutes. The institutes used to be administered by NTNF but on 1 February 1986 they were made autonomous in line with the Thulin Report of 1981. The outcome of a com-



mission set up to look at research and development along with industrial innovation, the report recommended that NTNF should be freed from administering the institutes to leave it more free to involve itself with planning and establishing research priorities. NTNF supports research carried out at these institutes, in industry and, to some extent, at universities. Inge Johansen, managing director of NTNF says that they wish to encourage "more innovation in industry and the transfer of technology from research to industry".

The NAVF, which like NORAS is attached to the Ministry for Cultural and Scientific Affairs, is the prime source of research grants for basic research in universities and regional colleges. It consists of various subcouncils for different research disciplines. The other research councils are attached to their corresponding ministries and promote research in the areas defined by their title.

The research councils have the task of coordinating research programmes within information technology, offshore technology, materials technology, biotechnology and aquaculture (fish farming) — the priority areas of research that were defined by the government in 1984–85. The White Paper "On Scientific Research in Norway" says that "these areas have been selected either because they spring

from new knowledge or they reflect new needs". A national programme for information technology R&D has been implemented this year and research programmes are currently under development for the other areas.

The Ministry for Cultural and Scientific Affairs has three additional priority areas, intended "to help society cope with the impact of high technology". These are: research on tradition and cultural dissemination (with the emphasis on Norway's heritage); organization, management and administrative systems; and health, environment and living conditions. The main responsibility for control of these programmes lies with NORAS and NAVF.

Of Norway's total public R&D budget (NOK 4.45 billion), most is dispensed by the Ministry for Cultural and Scientific Affairs, with the Ministry of Industry as the second biggest distributor. If the government has its way, spending on research will increase by about 13.2 per cent in 1988, with 42 per cent going to universities and colleges, 22 per cent to the industrial sector and 9.3 per cent to the agricultural sector. Fisheries and the increasingly supported environmental sector will each receive about 3 per cent of the total. The proportion earmarked for defence research (6.4 per cent) is relatively small, but considered sufficient because Norway is a member of NATO. □



Professor John Ugelstad of SINTEF was awarded the 1986 annual award of the Royal Norwegian Council for Science and Industrial Research for his development of the principles and methods of industrial production of monodisperse spherical polymer particles. When magnetized, these particles can be used for cell separation. For example, cancer cells in bone marrow can be separated from the normal cells by first adding mouse monoclonal antibodies that bind specifically to the cancer cells and then introducing magnetic particles coated with an immunoglobulin that selectively binds to the monoclonal antibodies. By passing the mixture through a magnetic field the cancer cells become trapped without disturbing the normal cells. Clinical trials of the method in bone marrow transplants are being carried out.

Universities

Going back to basics by network

ONE of the criticisms in the 1985 OECD report on Norwegian science policy was the imbalance between the emphasis on applied as opposed to basic research. According to Arnhild Hole, director-general of the Department of Science Policy at the Ministry for Cultural and Scientific Affairs, "there has been a famine in basic research at the universities for the past ten years", and it is one of the new Labour government's aims to encourage and revitalize basic research — and provide a broad base on which to build applied research.

Basic research in Norway is performed at the universities and regional colleges. There are four universities, of which the University of Oslo, established in 1811, is the oldest. The University of Trondheim was set up in 1946, and both Trondheim University (which includes the Norwegian Institute of Technology) and the University of Tromsø were established in the late 1960s.

The regional colleges are part of a regionalization policy followed by successive Norwegian governments in every area of national life. The aim is to stabilize scattered populations and counteract the attraction of towns, some of which are growing particularly fast because of activities related to oil production. Measures have been introduced to equalize incomes and working conditions and to decentralize industry. The regional colleges have been created since 1969 as centres of vocational training and higher education, and have since entered into the research arena. There has been criticism, particularly from the universities, that the regionalization of the research system is excessive, dissipating resources among establishments some of which have difficulty attaining a critical mass. The government's intended solution to the problem is to improve existing networks of communication between research institutes and to create new networks, says Hole. A university network has already been established (see below) and Norsk Data (a Norwegian information technology company) is working on a network to link the regional colleges.

Another problem is how to keep flesh on the bones of the country's regionalized system in the face of the 'brain drain' to Oslo, where much research and industry is located. Somehow incentives have to be provided to encourage people to remain in other regions of Norway. Also, two of the research councils (NTNF and NAVF) have scholarship schemes to encourage scientists to study abroad or elsewhere in Norway for a while before returning to their own institutes. And special scholarships to encourage people to stay in

Tromsø while giving opportunities for travel are included in a research support plan established for northern Norway with the aim of strengthening the University of Tromsø.

For Professor Nils Stenseth of the Biology Institute at the University of Oslo, one of the problems of basic research policy in Norway is that "a little money is given to everybody" as part of the philosophy of an egalitarian society. Once NAVF, responsible for funding much of university research, earmarks some of its budget for national priority areas, there is little left to be spread thinly among too many other research areas with the result that even good research projects are inadequately funded.

His other major complaint is about the tenure position at universities — it is a permanent position for life "with the free-

dom to do either anything or nothing". This has resulted in the generation gap so commonplace in universities, with ageing professors and no room for the young and talented people who should form the basis for the future. In an attempt to alleviate the situation, the government has created about 130 new research posts.

There are also problems lower down the scale. Norwegian undergraduates are funded by a combination of a stipend from the government and a loan from the state banks. The stipend is small, however, and according to Per Kofstad, vice dean of the Faculty of Mathematics and Science at the University of Oslo, "many students take part-time jobs to ease their financial situation". The high salaries offered by industry are then a powerful incentive for the deeply in debt student. There is, moreover, a shortage of qualified people choosing to teach, due to low pay and poor working conditions. All this, says Karl Schjetne of SINTEF, has led to a shortage in PhD students. □

SINTEF

Campus partners in technology

OF the several private research institutes in Norway the Stiftelsen for Industriell og Teknisk Forskning (SINTEF) or Foundation for Science and Industrial Research, is particularly interesting for its close links with a public institute of higher education. Because SINTEF and the Norwegian Institute of Technology (NTH) share the same campus at Trondheim, they can also share laboratories, equipment and specialist staff. Research staff at the institute participate in research projects in the foundation group whilst SINTEF staff often lecture at NTH and are very positive about the collaboration and its beneficial effects on their research activities, which include information technology, fish farming, civil and structural engineering, biotechnology, fluid dynamics and electronics.

The SINTEF group, which operates on a not-for-profit basis, consists of the SINTEF Foundation and three research companies: the Continental Shelf and Petroleum Technology Research Institute; the Norwegian Marine Technology Research Institute; and the Norwegian Institute of Electrical Supply. In 1986 the group's income was NOK 843 million, of which 63 per cent came from industry and most of the rest from the research councils.

Information technology is one of SINTEF's major interests, as befits the proud keeper of Norway's only supercomputer, a Cray installed in late November 1986. The universities, research institutes and parts of industry have access to the Cray. Finland is currently negotiating access. SINTEF sees itself as playing an important part in helping the Norwegian

information technology industry to become internationally competitive, says Karl Georg Schjetne, director of the foundation's computing centre, RUNIT. He feels strongly that more government support is needed for SINTEF in general, to increase capacity and encourage even greater collaboration with NTH.

Much of the work at RUNIT is concerned with database management, computer networks, expert systems and parallel processors. The network group helped to establish UNINETT, which interconnects Norwegian university data networks and links them to international research centres. The network became operational in 1987, and is part of the government's drive to improve communication between research centres.

In addition to RUNIT, two other groups give SINTEF a strong base in information technology. These are ELAB (the electronics laboratory) and the Division of Automatic Control. ELAB is closely involved with most industrially orientated R&D projects in electronics and telecommunications in Norway. It also performs some contract work for the European Space Agency in the field of satellite communications. The Division of Automatic Control works on such projects as a dynamic positioning for floating vessels (for example, oil rigs) and ultrasonic Doppler measurement of blood flow.

Research at SINTEF has to be geared to the demands of the clients it serves. Although this could be a cause of conflict with the NTH, which has no such constraints, few complaints are heard. □

Science-based industry

In search of a fertile future

NORSK Hydro is Norway's largest company and is 51 per cent state owned. With a turnover of NOK 40,000 million and about 31,000 employees, it has come a long way since its origin as a regional producer of fertilizers. Not only has Norsk Hydro joined the ranks of world leaders in fertilizer production by the acquisition of large European companies and investments in new production units, it has also developed major interests in light metals (magnesium and aluminium), petrochemicals, and oil and gas production.

Norsk Hydro's production of oil and gas in 1986 amounted to 4.3 million tonnes of oil equivalent, 80 per cent of which was gas. Rolf Prydz, head of the oil and gas group, says that the company "has been partner as operator or non-operator for some of the most interesting discoveries on the Norwegian continental shelf". The fields at Ekofisk and Frigg account for the greater part of the company's petroleum production. In 1986 research and development in the oil and gas group cost the company NOK 248 million. It is essential, says Prydz, to increase investment in research aimed at profitable oil and gas production even at a price as low as US \$15 a barrel. Research efforts include improving knowledge of geological conditions in oil fields, enhancing oil recovery and projects for the design of less expensive constructions for the development and operation of oil and gas fields offshore.

The rapid development of the oil sector and its impact on the Norwegian nation has been one of the major events of recent years. The oil and gas fields on the continental shelf of the North Sea constitute some of Norway's most important natural resources. The first production licences were granted in 1965 and output began in 1971. It rose substantially in the second half of the 1970s when pipelines to Germany and Scotland were built. Norway is now one of Europe's largest exporters of energy products. These represent nearly 30 per cent of the country's total exports. Because of oil, national income has been growing fairly steadily and there is little unemployment despite the fact that the world economic recession has reduced outlets for other Norwegian products.

No industrial position is unassailable, however, and flexibility is needed to respond to the needs of a changing market. The government has designated five priority areas for industrial research in the hope of stimulating rapid growth in other export earnings over the next ten to twenty years.

To meet such demands, Norsk Hydro set up Hydro Innovation in 1985, to explore potential business areas. The new venture concentrates on biotechnology

(including fish farming), materials technology and offshore technology. Research is being carried out on processing fish waste as fish entrails contain a number of compounds of interest, such as enzymes, water-soluble nutrients and fat compounds. The use of these products in the medical, food manufacturing and chemical industries is being investigated. Light metals, polymers and ceramics are being studied since composite materials with improved qualities of strength, corrosion and heat resistance are being sought, particularly for the automobile industry. There is a drive to reduce harmful emissions, fuel consumption and improve an engine's efficiency. □

Fish farming

Moving from salmon to flatfish

Fish farming has been so successfully developed in Norway that the pioneers of industrial salmon farming, Norsk Hydro have become victims of their own success. Worried that large-scale salmon farming would deprive the traditional fishermen of their living, the government has ruled that licences are required for salmon farming and has granted them mainly to the fishermen on the country's coast. Norsk Hydro has formed subsidiaries in Scotland, Ireland and Iceland.

Most commonly the salmon 'farms' comprise cage structures with a number of separate closed nets linked in a variety of ways to make a complete plant. Such plants demand a relatively small investment and are popular with the people living along the coast of Norway who have set up the salmon farming industry.

Various problems arise with the use of these cages. The temperature and salinity of the water varies in the upper levels depending on season and rainfall. In winter the temperature falls below 4°C which is too low for salmon to thrive. Plants located in places with low water

Science parks

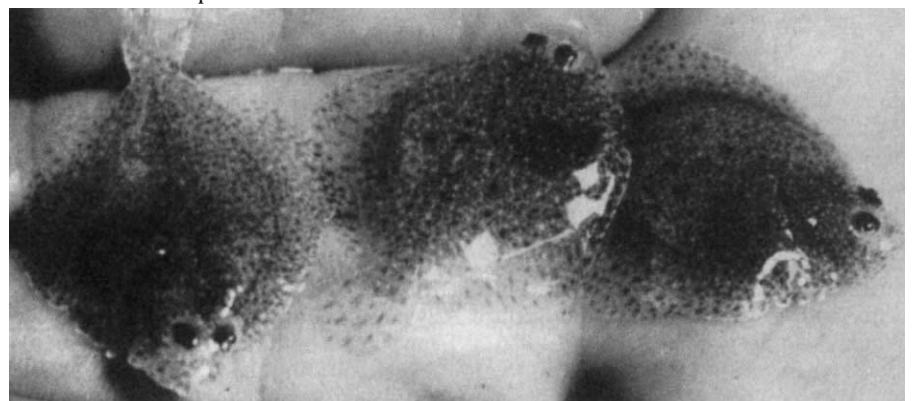
RESEARCH foundations set up around the universities are fast gaining popularity in Norway. For example, one is being established in Oslo and will be run along the lines of the science park at the University of Cambridge in the United Kingdom. It will be financed by a shareholding company involving industry, Oslo University and ten private research institutes

The government is keen to promote links with industry, though it is emphasized that the foundation in Oslo will not be industry-led. All parties will have a say in its running. Basic research and industrial research will each represent 40 per cent of the foundation's efforts. The remaining 20 per cent will be 'innovative' research. A comparable development is under way at the University of Bergen. □

exchange have problems with feed waste and excrement accumulating below the cages and impairing the water quality. There is increasing competition in salmon farming and therefore there is a trend towards more industrialization with all stages being monitored and controlled.

In salmon farming, research is concentrated on improving the technology. Particular attention is being paid to improving the binding agents in the feed to increase the nutritional value, the physical environment of farming facilities, and development of new designs of plant. Also, instrumentation is being developed to determine the size and number of fish and to monitor behavioural patterns.

There are also potentially good markets for farmed marine flatfish such as halibut and turbot. SINTEF and companies such as Norsk Hydro are directing a comprehensive research effort in this area. One problem is that halibut and turbot are very sensitive to environmental conditions. In addition marine flatfish larvae are much smaller than salmon and require live feed. □



Turbot larvae are small — for turbot and halibut Norsk Hydro are carrying out research into controlled spawning, improved egg quality and how best to provide the larvae with live feed.

Environmental research

Acidification under attack

CAUSTIC exchanges on the topic of acid rain have been a feature of relationships between 'exporting' countries, such as the United Kingdom, and 'importers' like Norway and Sweden. But while, or perhaps because, the politicians bicker, the scientific effort to understand the causes and consequences of environmental acidification is on the increase, not least in Norway.

During the past few decades, acidifications of lakes and rivers in northern Europe has resulted in severe losses of fish, most notably the brown trout and Atlantic salmon. However, the fact that acidic waters are found in areas with acidic soils has led some scientists to cast doubt on the role of atmospheric acid deposition in acidification and to argue that it can be accounted for by changes in the terrestrial ecosystem. This controversy has raged over the past fifteen years or so stimulating much research into the effects of atmospheric acid deposition on soils, lakes, fish and trees.

Detailed studies of soil chemistry have provided convincing evidence that acid rain is the cause of the transfer of acidity from soils to their surface waters and thence to lakes and rivers. One of the critical questions is how quickly and to what extent the damaged ecosystems respond to changes in atmospheric acid deposition. The water quality changes of interest occur over years in natural systems, so direct observation of the dynamics of these changes requires lengthy time series data.

The five-year international RAIN (Reversing Acidification In Norway) project is looking at links between deposition and freshwater acidification. At Sogndal in north-west Norway, where there is no acid rain, experimental acidification of the precipitation is allowing the effect of acid rain on a previously clean environment to be monitored. At Risdalsheia in southern Norway, which is one of the worst-afflicted areas, acid precipitation is excluded from a headwater catchment by means of a roof and is then purified to a pre-acid condition and sprayed onto the catchment. Richard Wright of the Norwegian Institute of Water Resources (NIVA) is a driving force behind this project and says that the results "show that the chemical changes caused by acid deposition are largely reversible".

Much of the research on soil, surface water and fish forms part of the Surface Water Acidification Project (SWAP), a joint venture of the Norwegian Academy of Sciences and Letters, the Royal Swedish Academy of Sciences and the UK Royal Society, and supported by the UK

Central Electricity Generating Board (CEGB). The focus is on the biological, chemical and hydrological factors that are important in determining whether water quality is acceptable for fish, and on the effects of reduced deposition of anthropogenic sulphur on surface waters.

Considerable work on the modelling of sulphur and nitrogen deposition has been performed at the Norwegian Meteorological Institute in Oslo. This work was first started around 1972 as part of an OECD programme on acid deposition in Europe and has subsequently developed under the sponsorship of the United Nations EMEP programme. All participating countries have to give total annual emission data for the relevant pollutants. According to the institute's Professor Anton Eliassen, all Western European countries give more-or-less gridded data on emissions (the grid scale of the model used is 150 km) but "some have difficulty in agreeing on emission distribution".

A new model for nitrogen oxide emission has been developed this year in collaboration with Øystein Hov of the Norwegian Institute for Air Research. Comparisons between measured and calculated data generally show good agreement although there is a systematic underestimation of nitrate in precipitation. Nitrogen oxides are transported at least as far as sulphur oxides with deposition differences reflecting the geographical distribution of sources.

Since Norway, like other Nordic countries, has a substantial industry based on wood, there is particular concern over the effect of acid rain on trees, a matter of considerable controversy among Norwegian scientists. Opinion is divided among those who believe that forest damage is caused largely by climatic change, those who feel that changes in soil chemistry are the key, others who attribute the damage primarily to acid deposition on the needles and those who believe it is due to a combination of all these.

Eliassen says that "there is a great deal of tension among forestry scientists in Norway". In part this stems from an SNSF project set up by the Norwegian government in 1972. One of the underlying assumptions was that acid rain was responsible for forest damage. Some scientists seem to have resented this assumption, over-reacted and set themselves to prove that acid rain was not the major cause. By contrast, there is little dissent among Swedish scientists who share the view of Jan Nilsson of the Swedish Environmental Protection Board that acid deposition has caused a dramatic change in soil chemistry in southern Sweden which has undoubtedly stressed the trees. □

'Green' ministry goes critical

THE 'critical load concept' has become an oft-used phrase in acid rain policy. The critical load, defined as the highest deposition that will not cause chemical changes that will lead to long-term harmful effects on ecosystems, was accepted as the basis for negotiations in Geneva on transboundary pollutants in November 1986. For sulphur, the critical load is 3–5 kg per hectare per year for the most sensitive ecosystem. For nitrogen, it is 10–20 kg per hectare per year. Current research in Sweden is planned to determine the critical loads for different types of ecosystems. Negotiations based on critical loads have yet to be carried out.

Per Bakken of the Norwegian Ministry of the Environment says that the critical load concept is "more or less agreed" among the Nordic countries, and Norway is aiming to



Per Bakken: can his ministry "save the rain"? reduce emissions accordingly.

The Norwegian government will be applying stricter standards for all types of heavy truck in the 1990s and catalytic converters will be fitted on all new cars from the beginning of the 1990s in an attempt to reduce their nitrogen oxide emissions. But the many boats in Norway that are sources of nitrogen oxide emissions create a more serious problem because people do not often buy new boats and it is difficult to convert them to comply with regulations.

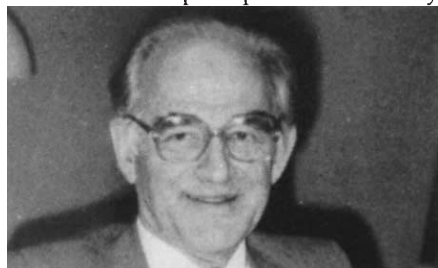
Sweden plans to decrease sulphur dioxide emissions by 65 per cent and nitrogen oxide emissions by 30 per cent between 1980 and 1995. All new petrol-driven cars will have to be fitted with catalytic converters to reduce emissions from 1989 and many new cars are already so equipped. But both Norway and Sweden feel strongly that other countries, especially Britain and Poland, need to do more to control their 'exports'. □

Policy

Keeping a lead in R&D

WITH its glossy cover and text illustrations, and published in word-perfect English, there is an immediate temptation to approach the 1987 Swedish government research bill with caution. What, one wonders, is it trying to sell? The answer is a high-grade product of the consensus politics that dominate Swedish affairs.

The bill, which has passed through parliament, lays out the policy, priorities and budgetary increases for the next three years of government-financed research and development, and is the third in a line of research and development bills in the past five years. Perhaps this is the kind of activity one would expect from a government that runs the most R&D intensive country (with a budget that is about 2.7 per cent gross domestic product) in the OECD. And perhaps that is why



The governmental advisory board is now listened to, says Bert Bolin.

Swedish science policy is both coordinated and created within the cabinet office of the prime minister.

Indeed, the current prime minister, Ingvar Carlsson, takes personal responsibility for the coordination of science and technology policy, as he did when deputy to Prime Minister Olaf Palme before his assassination in February 1986. Carlsson also chairs the Government Research Advisory Board which has regained its former importance since the Social Democratic Party returned to power in 1982, according to board member Bert Bolin, who also acts as personal advisor on science to the prime minister and is Professor of Meteorology at the University of Stockholm.

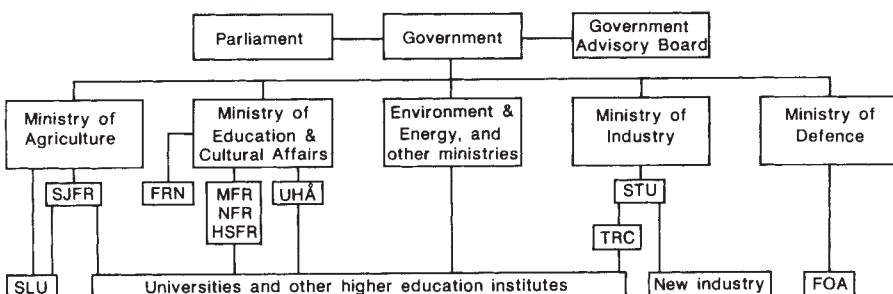
Apart from Carlsson and Bolin, the board includes the ministers of education

and industry together with six scientists chosen in their personal capacity. There are four meetings a year that deal with topical matters and four that consider longer-term issues, which are not necessarily science-related. Biotechnology has, of course, been on the agenda but so has the relationship of religion to society. And although the board is not decision making, it clearly influenced recent decisions both to channel more government funds to the basic sciences that underlie biotechnology and to finance two new theology chairs.

Government research policy, as it is enacted in the bill, has to take into account the demands of twelve ministries, among which three are the most demanding. Of the SEK 11,350 million government R&D budget for 1986–87, 30 per cent was channelled through the Ministry of Education and Cultural Affairs to support higher education institutes and three research councils — the Medical Research Council (MFR), the National Science Research Council (NFR), and the Council for Research in Humanities and Social Sciences (HSFR). Also funded by this ministry is the Council for Planning and Coordination of Research (FRN) which cooperates both with the research councils and with sectorial bodies.

A further 16 per cent went to the Ministry of Industry and its National Board for Technical Development (STU), which incorporates a Technical Research Council (TRC) and 25 per cent was given to the Ministry of Defence which supports the National Defence Research Institute (FOA). Next in line is the Ministry of Agriculture, which takes 6 per cent of the budget and runs both the Swedish University of Agricultural Science (SLU) and the Swedish Agricultural Research Council (SJFR).

As in most developed countries, the research councils hold the key to success in academic research. It is not impossible to continue on a small scale with university funds or even on a large scale with external support for applied research but to mount an effective programme of basic scientific research it is necessary to obtain



The Swedish way of controlling higher education and research. Sectoral research institutes are notable for their absence.

Outsiders look in

SENSITIVE to the possible shortcomings of relying solely on internal evaluation in a country as small as Sweden (which has a population of 8.5 million), the NFR supplements its standard peer review of grants with a scheme of regular external evaluations of the research programmes it supports. Typically, four overseas scientists, accompanied by an NFR rapporteur, undertake a whistle-stop tour of most of the grant holders, spending an hour with each and then writing a frank appraisal of their work, which is later published. Sample comments on two individuals from a 1986 evaluation of physical chemistry read: "This is a classic example of a case where an initially good idea, worth a try, and a committed man should have been redirected or terminated earlier, in his own interests" and "the Evaluation Committee . . . advise that judicious exercise of the big stick which goes with office can sometimes work wonders".

Professor Carl Nordling, recently appointed secretary general of NFR, says that the evaluations are not the only parameter but undoubtedly the most important by which anyone is judged. Needless to say, they are also compulsive reading material for other academics, and influential in private foundations and in ministries. □

a grant from MFR, NFR or SJFR. "Our funds are small but strategically very important", says Henry Danielsson, who as MFR secretary, distributes some SEK 200 million a year, about 10 per cent of government spending on medical research.

Naturally he would prefer to have a larger slice of the cake, but is unlikely to do so while the country maintains its very pluralistic approach to research. Some medical research, for example, is commissioned by at least three ministries apart from Education and Cultural Affairs.

An equally strong policy, however, ensures that most mission-orientated (or sectoral) research is carried out in universities (and other higher education establishments) rather than in separate institutes set up by the relevant ministry. Such institutes, says Bolin, are notoriously slow at adjusting their research to circumstances, particularly because they tend to have no flow-through of graduate or postgraduate students. By concentrating all resources on the universities a critical mass is more likely to be achieved in a small country, he adds.

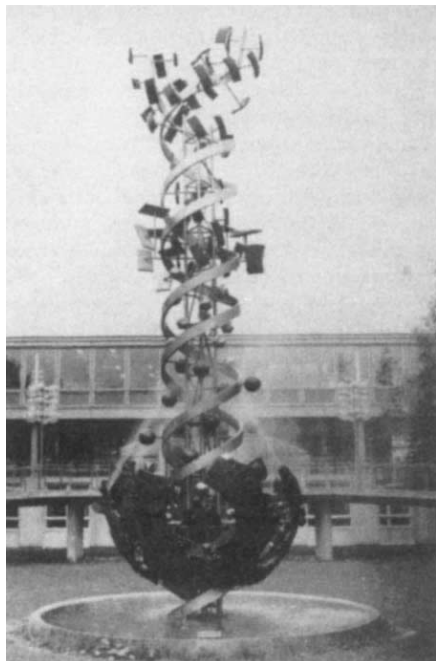
Widespread criticism, nonetheless, of the value of short-term sectorial research has led to more stringent evaluation procedures. For example, overseas evaluation is employed by STU. There is also a marked move towards persuading the sectorial funding agencies to support academic posts, from postdoctoral positions to 'extra' chairs (see page 340). □

Uppsala University

FOUNDED in 1477, Uppsala University is the oldest in Scandinavia and boasts such scientific luminaries as Carl von Linné — better known as Carolus Linnaeus — Carl Wilhelm Scheele, Olof Rudbeck, Anders Celsius, Anders Ångström and half a dozen Nobel laureates. Like other ancient universities, it is also blessed with the ownership of some estates that used to provide its main income but now contribute a small, but useful, 2 per cent of the total.

With 15,000 undergraduates and at least 2,500 postgraduates, the university offers the full range of academic subjects and has the largest mathematics and natural science faculties in the country. It also has a vast Biomedical Center (BMC) built between 1968 and 1984.

Occupying about one third of the floor space of the whole university, the BMC



The DNA fountain that symbolizes much of the research at Uppsala's Biomedical Center.

houses under one extended roof about 1,300 staff drawn largely from the preclinical departments of the medical faculty. In addition, it contains the faculty of pharmacy, some departments from the Swedish University of Agricultural Sciences, a government veterinary testing centre and a recently created branch of the Ludwig Institute (a privately-financed cancer research organization).

The advantages of this conglomeration include the ease of access to like minds and the possibilities of sharing expensive equipment. Central facilities are under the control of BMC's director, Professor Lars-Olof Sunderlöf, but he has no control over the affairs of the departments that share BMC's space. "Contractions and expansions have happened", he says, but he is powerless to insist on any changes. □

Universities

Survival after reform

IT is ten years since higher education in Sweden underwent a major reform that left its mark on the country's six universities, in Stockholm, Uppsala, Göteborg, Lund, Linköping and Umeå, which are supplemented by the more numerous university colleges and by postgraduate training institutes, such as the Royal Institute of Technology in Stockholm. Perhaps the two major changes were the opening up of higher education to a wider sector of society and the decentralization of much decision making.

In connection with the reform, the former Office of the Chancellor of the Universities was replaced by the Universitets- och Högskoleåmbetet (UHÅ) or National Board of Universities and Colleges, which is the administrative organ of the Ministry of Education and Cultural Affairs.

In many respects, says UHÅ's Camilla Modéer, the decentralization measures have given the universities much more flexibility than before in how they spend the money they are centrally granted for education and research. But a striking reminder of the relative lack of autonomy of the universities is that the creation of a new chair still needs parliamentary approval. There is also a device of creating an 'extra' chair, which is within a university's power but for which no extra central funds are available. Increasingly, they are likely to be paid for by sectorial research funds or private donations. In theory the 'extra' chairs are not permanent; in practice, it is recognized that universities will be obliged to pick up the bill if other sources dry up.

While the universities welcomed their increased autonomy, they have had mixed feelings about the reform that opened them up to non-traditional applicants, especially those who wanted a second chance of a higher education, having opted out in favour of work on the first occasion. The difficulties have been greatest for medical schools. At the Karolinska Institute in Stockholm, the mean age of students rose from 20 to 28 as a cohort of ageing would-be medics gained belated entrance. But, says the institute's dean, Professor Sten Orrenius, this intake was not very successful. Many of them were unrealistic about the time and effort that goes into medical studies, and had hoped to hold at least a part-time job while studying in order to support their families. By now, the mean student age is back down to 22.

Johnny Andersson, head of administration at Uppsala University says the problems are exaggerated at medical schools, and that the reform is justified as a measure of social democracy. "It is particu-

larly important to give people a second chance in a small country that needs to exploit every educational opportunity", he says.

A bone of contention for the students themselves is the extent to which they have to repay their educational costs. Whereas the loan scheme, when introduced in 1964, applied to only 75 per cent of the costs it has now risen to 95 per cent, more by evolution than design. Moreover, like students everywhere, they want more money. A new proposal that stands a good chance of becoming law will increase the total grant slightly and reduce the loaned proportion to 75 per cent but make repayments faster.

For postgraduates, the major problem is how to complete their PhD, or its equivalent, in the five years available. This seemingly generous timescale is offset by the fact that postgraduates are not supported by a grant but are employed by the university, in return for which they have to teach for 2 or 3 months a year. The main concern for postdocs is where the next job will come from. Options are limited by the strictly enforced rule that universities will only employ any postdoc for one 4-year research assistant. Thereafter, it is necessary to find either external support, for example from a research council, or one of the occasional tenured university posts that become available.

As for university staff, their salaries are rather low in European terms and staff in the predictable areas of science are increasingly prey to industrial overtures, says Andersson. As a result a long-standing resistance to differentials in professional salaries is, at last, being overcome.

Surprisingly, there is little problem of a 'brain drain' overseas. One financial attraction of an academic position is that any discovery or invention belongs solely to the individual and not to the institution.

For the universities as a whole, a small but real increase in direct funds, via UHÅ, has been guaranteed for the next three years; against global trends, however, most of it will be spent on the humanities.

Where science departments will benefit directly is in the provision of extra funds for new equipment. For some years there have been special funds for this purpose within the research councils and university chancelleries, as well as access to sizeable grants for heavy equipment from the Wallenberg Foundation. Now, apparently under pressure to donate or be taxed, the highly profitable Swedish banks have agreed to make SEK 200 million available over the next three years for the purchase of equipment. All told, the equipment funds currently amount to about SEK 400 million a year. □

Karolinska Institute

SWEDEN'S biomedical community likes to remind visitors that of the *Science Citation Index's* "1,000 Contemporary Scientists Most-Cited 1965–1978", a disproportionate 42 are Swedish and that all of these are from the biomedical sciences. The Karolinska Institute in Stockholm can lay claim to half of the 42.

Although effectively acting as the medical and dental schools of the University of Stockholm, the Karolinska Institute maintains a fierce independence from the university, whose existence it predates. Since 1977, all government-run medical and paramedical education in the Stockholm region has been carried out by the institute's 850 teaching and research staff. These are divided into 140 professorial departments which are very unequally divided between a north and south campus.

The former, situated just north of the city centre, has the advantages of size, power and tradition. It is where, for example, the institute's tradition of medical chemistry was begun by Jöns Jacob Berzelius, who discovered the elements Se, Si and Th, and introduced the system of symbols by which they are here identified. More recently the tradition has been continued by Professors Sune Bergström and Bengt Samuelsson (the institute's current president) with their discoveries of new biologically-active chemicals related to prostaglandin, itself originally discovered, at the institute by Ulf von Euler in the 1930s.

The south campus at Huddinge University Hospital, some 15 kilometres from the city centre, dates only from the 1970s. It houses the dental school and some medical departments including the department of medical nutrition, an unlikely place to find such an indefatigable supporter of molecular biology and biotechnology as its head, Professor Jan-Åke Gustafsson.

With financial assistance from Stockholm County Council, keen to breathe new life into the economically underdeveloped southern parts of the city, Gustafsson has created the Center for Biotechnology, the 100 staff of which will pursue basic research but provide "a broad interface with industry". Within two years the centre, of which Gustafsson is managing director, will be in a new building, Novum.

A third occupant will be a new biotechnology company, KaroBio, with which Gustafsson is also involved. It will focus on infectious and bone diseases as well as steroid hormone agonists and antagonists and is financed largely by Swedish institutional investors but also by California Biotechnology Inc. The foundation of KaroBio somewhat counteracts recent decisions by Pharmacia and Astra — Sweden's largest pharmaceutical companies — to start biotechnology offshoots in California and India, respectively. □

SIPRI

Spawned by unbroken peace

CRITICS of the Stockholm International Peace Research Institute (SIPRI) used to call it an organ of the 'left'. But now, and particularly in Swedish 'peace' circles, it is criticized as a "NATO institute", says its latest director Dr Walther Stützle, who until a year ago was undersecretary for defence, planning and policy for the West German government.

All of which goes to show how slippery is the definition of peace research, not least for an institute that was founded in 1966 to commemorate 150 years of unbroken peace in Sweden. It was a former prime minister of Sweden, Tage Erlander, who suggested the institute, and a Royal Commission that designed it. And it is the Swedish parliament that still provides almost all the funds — SEK 18 million this year.

Under Stützle, SIPRI's focus of research is on military realities and arms control possibilities, with an emphasis on five areas. Two of these are the traditional SIPRI topics of military expenditure and arms trade, on which the institute keeps a unique databank. The extensive information gathered in both areas is included in SIPRI's bread-and-butter project, its yearbook, which now runs to some 500 pages. Breaking with tradition, the 1987 yearbook contains no estimate of the Soviet defence budget: no reliable estimate can be produced, says Stützle.

The other three broad areas of research are the US–Soviet strategic relationship, European security and arms control, and weapons technology and arms control. Within these fields some studies are devoted to issues that are of immediate relevance to policy, while others deal with issues that are more in the background. It is much harder now than it must have been in 1966 to carve out a niche for SIPRI, says Stützle, because of the burgeoning of peace research. The institute's location in a neutral country remains an advantage when it comes to persuading Eastern bloc delegates to the small seminars that are another of SIPRI's activities; there is an ambitious plan, for example, to bring together actively-serving experts on threat

assessment from the military establishments of the east and west.

Chemical and biological warfare (CBW) issues have always been on SIPRI's agenda. They are now under Johan Lundin, a physiologist previously employed at the Swedish National Defence Research Institute, and an erstwhile Swedish delegate to the CBW negotiations in Geneva. One aim of his group's research is to define verification systems that would be acceptable to the chemical industry, given the need for normal commercial and technical secrecy.

One of Stützle's favourite projects is an example of "what if?" research, an



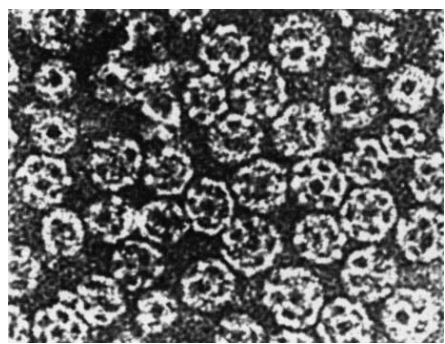
SIPRI director Walther Stützle — treading the delicate line between the right and left of peace.

approach that SIPRI can afford to take. The question is what would be the effects both for Europe and the United States if US armed forces were to be removed from Europe. This project is supported by the MacArthur Foundation in Chicago, and Stützle hopes for more external support to counter a decreasing budget in real terms from the Swedish parliament. Like other European research directors he finds it impossible to compete with salaries that can be offered the most prestigious US universities, and yet is loath to settle for less than the best researchers.

Does SIPRI have any connection with the Nobel peace prize? No, says its director, except that it hopes to win one, "but that would only happen long after my time!" □

An ISCOM from SLU

Bror Morein of the Swedish University of Agricultural Sciences (SLU) at the Biomedical Centre in Uppsala has pioneered the development of vaccines based on immuno-stimulating complex (ISCOM) particles. The matrix of the cage-like particles is composed of Quil A, which is extracted from the bark of a South American tree. Antigenic proteins attached by hydrophobic interactions to the surface of the matrix evoke a strong immune response.



Deep gas drilling divides the experts

COULD the answer to Sweden's lack of indigenous fuels lie 7.5 kilometres under a meteorite crater in central Sweden? When the drilling stopped at 6.3 kilometres for lack of money at the end of August some evidence had been obtained to support the controversial idea of Cornell University's Professor Thomas Gold that hydrocarbon gas deposits of non-biological origin are waiting to be found at such sites.

Drilling of the Gravberg borehole began in July 1986 under the leadership of Tord Lindbo of the state-owned power board, Vattenfall, which joined private investors in forming the company Dala Djupgas Provbörningar KB to back the project. Drilling at first went better than expected but then suffered various setbacks. While not discovering commercial quantities of



The deep gas drilling rig at Gravberg.

gases, a variety of hydrocarbons had been found in significant quantities. Critics maintain that these are artefacts of the drilling but Gold and Lindbo disagree, pointing in particular to the way in which the various gases track each other with depth. "There is no other circumstance that could have caused the correlations than that of their correlated entry into the wellbore from the formation", asserts Gold.

There is a particularly interesting seismic reflector at 7.2 kilometres, says Lindbo, who is keen to drill through it and optimistic that the private investors will pump in the extra SEK 20 million that would be needed to support the further three months of drilling. The scientific case for continuing is strong but the backers will need to be convinced that there remains a possibility of finding commercial quantities of gases. A considerable number of experts are being asked to assess the chances and, says Lindbo, commercial consultants are more optimistic than academics. A decision is expected by January. □

Energy

In the cold without nuclear power?

SWEDEN was once sensible enough, from the British and Japanese perspective, to drive on the left-hand side of the road but the rarely used device of a public referendum led it to fall into line with its Nordic neighbours. The latest referendum, in the year following the accident at the US Three Mile Island nuclear reactor in 1979, resulted in a parliamentary decision to shut down by the year 2010 the last of Sweden's 12 nuclear reactors, which produce 15 per cent of the country's total energy supply and half of its electricity.

Whereas the assumption in some circles was that this decision would later be overturned, the Chernobyl accident, from which Sweden received more radiation exposure than most countries, put paid to any immediate prospect of a change in public opinion at least. Therefore the country's energy policy and research is being increasingly driven by the prospect of survival without nuclear power, not least because 2010 is "almost tomorrow" for new energy investments, in the words of Leif Brandels, head of the planning office of the Statens Energiverk or National Energy Administration (NEA).

The NEA is the national coordinating energy authority in Sweden and is answerable to a ministerial department with a title of unusual significance — the Department of Environment and Energy. That title assumes particular relevance when it is appreciated that the energy planners have their hands tied regarding any expansion of Sweden's other main indigenous source of energy, hydroelectric power. On environmental grounds, parliament has declared that the hydroelectric potential of four northern rivers must stay untapped.

The problem is exacerbated by the country's almost complete lack of coal, natural gas or oil. For obvious reasons Sweden would be very reluctant to reverse its decreasing reliance on oil since the early 1970s achieved in part by the emergence of nuclear power.

So what will substitute for nuclear power, which currently generates nearly 70 terrawatt-hours of energy? Most likely, says Brandels, at least 20 TWh will have to come from imported natural gas, with Norway and the Soviet Union as the two most likely suppliers, unless the highly speculative deep gas drilling project (see left) proves successful. And efficiency measures can probably reduce demand by a further 20–30 TWh, mainly by cutting down the extent to which electricity is used for domestic heating.

With these needs in mind, and because Sweden suffers a cold climate, it is not surprising that the country is one of the highest spenders *per capita* on research

and development in energy, although the 1987–90 budget will be considerably less than the SEK 1,200 million spent by government on energy research and development in 1984–87. About half of this was related to energy supply and so was channelled through NEA in several areas.

Highest priority was given to combustion technology research with attention divided between the combustion processes themselves and the ways in which their toxic emissions can be minimized. Peat was the next priority since its use as a fuel has revived in the past decade. Research now concentrates on extraction and processing methods. Other NEA programmes are concerned with wood, wind and fuel consumption. □

After Chernobyl

AN impressive 300-page report with that title was completed at government's request within six months of the Chernobyl accident (26 April 1986). It investigates the consequences of Chernobyl for Swedish energy policy, nuclear safety, and both radiological and environmental protection, and it has two major conclusions.

The first is that there is no need to modify the technical risk assessments of the Swedish reactors or their design. A process, already begun, to upgrade protection measures against large-scale accidental release of radioactivity from the country's nine boiling-water and three pressurized-water reactors should continue, it concluded, but no extras measures are called for.

Second, it would not be possible to phase out nuclear power within ten years, rather than by 2010, without a considerable increase in the cost of electricity. A ten-year phase out would rely largely on the generation of conventional coal-based power with sophisticated techniques to reduce sulphur and nitrogen oxide emissions, since extra hydropower is effectively ruled out, oil is too risky and time is too short to adapt to large-scale use of natural gas.

By sticking to 2010, there would be time to convert to gas but also the possibility of exploiting new technology that is in an advanced stage of development. In particular, gasification and pressure-fluidized bed combustion of coal are considered techniques of considerable promise, particularly in environmental terms. Wind power could also contribute; Sweden has two land-based plants in operation but it would need several thousand off-shore plants to replace current nuclear power production.

The government's response, not surprisingly, has been to hold to its original plans, estimating that the first of the nuclear plants could be closed in 1993–95. □

Nobel prizes

No expense or effort spared

As this year's Nobel prize winners head for the award ceremonies on 10 December — the anniversary of Alfred Nobel's death in 1896 — they will already be used to the fame and aware of the fortune that the prizes bring. What they will not know is just how much time and money has been spent on their selection, reflecting the seriousness with which the whole business is treated in Sweden (and Norway for the peace prize).

And business it is for the Nobel Foundation, which was established under the terms of Nobel's will as the recipient of most of his fortune. The prime purpose of the foundation is to invest its capital in such a way as to finance the five prizes, each of which was SEK 2.175 million this year. Astonishingly, another SEK 10.9 million will have been spent this year "in the work of assessing the candidates", which involves some 200 people, and some SEK 2.5 million on the presentations and associated events.

Alfred Nobel, whose fortune came largely from the invention and exploitation of dynamite and other explosives, stipulated that there should be prizes in physics, chemistry, physiology or medicine, literature, and peace. The economic sciences prize was added in 1968 at the request, and with the financial backing of, the Bank of Sweden, which was then celebrating its 300th anniversary. Stig Ramel, executive director of the Nobel Foundation in Stockholm, says that all other overtures to add to the number of prizes have been, and will continue to be, declined. Were that not the case there might well be Nobel prizes in, for example, mathematics, ballet, music and architecture.

One of Ramel's goals is to restore the original value of the prize, which is currently only about two thirds as valuable as when Röntgen and Van't Hoff, among others, became the first Nobel laureates in 1901. Until a government decision in 1953 the foundation's capital could only be placed in 'safe' securities and had, as a result, lost some two thirds of its value. By the end of 1986, its assets of around SEK 800 million were close to their original value, although they have been dented by recent stock market problems.

But it is the kudos attached to the prize, rather than its monetary value, that remains its major attraction to potential winners. In any case, the prize awarding bodies are at great pains not to err in their judgement and, at least in physics and chemistry, claim never to have become embarrassed by their choices.

The selection procedure begins in the preceding September with a request for nominations. This is sent to, among others, all previous laureates, who tend to excel at

their task and particularly at nominating outside their own field, says Jan Lindsten, who occupies the key post of secretary of the Nobel Committee for Physiology or Medicine at the Karolinska Institute, where he holds a chair in medical genetics.

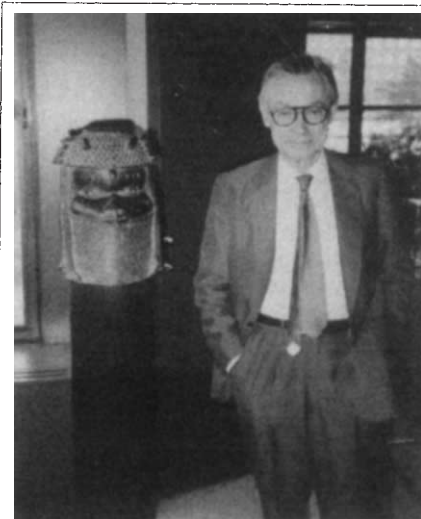
The 200 or so nominations for each year's physiology or medicine prize are considered by a committee of six which can appoint ten additional *ad hoc* members and can commission external reports on candidates whose field of work lies outside the knowledge of committee members. A 2–3 page assessment of each serious candidate is first prepared, and then 50 are selected for more thorough investigation. At the end of August the 50 are gradually whittled down to the candidates (up to three for each prize) that the committee will recommend. Occasionally a committee is split and makes two alternative recommendations. In any case the final word lies with the 50-strong Nobel Assembly of the Karolinska Institute. A similar procedure, although different in detail, operates for the chemistry and physics prizes. They, however, are awarded by the Royal Swedish Academy of Sciences, whose 275 or so members have the final say once the separate committees for each prize have made their recommendations. The literature prize is handled through the Swedish Academy and the peace prize by a committee appointed by the Norwegian parliament, in accordance with Nobel's wishes.

While there were several occasions in the first 40 years of the prizes when awards

Not the Nobel

THE Nobel Foundation may be unwilling to add to its prizes but the Royal Swedish Academy of Sciences, which awards two of them, is obviously not. For the past five years it has been awarding the Crafoord prize in areas of science that are effectively excluded by Nobel's will. The annual prize, "of a sum exceeding one million Swedish crowns", is awarded by rota to mathematics alternating with astronomy, geosciences, and biosciences (with particular emphasis on ecology). Along with each prize research grants are awarded to Swedish scientists working in the same discipline. In addition, rheumatoid arthritis receives a research grant every third year and a Crafoord prize when progress justifies it.

Both prizes and grants are financed by a fund established in 1980 by a donation from Anna-Greta Crafoord and Holger Crafoord, a Swedish industrialist and founder of AB Gambro, a medical instrument manufacturer. □



Professor Sune Bergström, 1982 Nobel laureate in physiology or medicine, and a Benin bronze, part of the collection of precious objects that Georg von Békésy, 1961 Nobel laureate in physiology or medicine, bequeathed to the Nobel Foundation.

were reserved, apparently for lack of an appropriate candidate, this has not happened since the end of the Second World War. Nor could it happen now, says Gösta Ekspong, chairman of the Nobel Committee for Physics and a professor of physics at Stockholm University. Indeed, he admits that there are some worthy candidates who will never get a prize simply because there are not enough to go round. Occasionally the upper limit of three winners a year has made it impossible to award a prize for a particular discovery, he adds.

Those involved with the prizes recognize that such accolades are not wholly beneficial for science but argue that the good aspects outweigh the bad. There is no doubt about their importance to the Swedish science community. Professor Sune Bergström, a 1982 laureate and senior statesman of the community, says that from their beginnings the prizes stimulated Swedish science through the contacts they brought in their wake.

But the extent to which the prizes are taken as a measure of national esteem is deplored. They are not the Olympic Games, says Ramel, recalling, nevertheless, how César Milstein had to be deemed to be Argentinian — his country of origin — until midnight on 10 December 1984 and British — his adopted nationality and place of work — thereafter, in order to allow the relevant ambassadors each to attend part of the ceremonies without overlapping, a diplomatic impossibility at the time. And Ekspong points out the irony of the situation in which prizes that the awarding bodies were at first reluctant to handle, because it seemed unpatriotic that they were not reserved for Swedes, are so subject to national appropriation. □

Industry/university

Takeovers and interfaces

FOR its size, Sweden has a remarkable number of large and internationally-known science and technology-based companies, which are in the habit of investing heavily in research and development. A new habit, which characterizes the past two years, is for these companies to grow by means of takeovers or mergers. For example, ASEA, Sweden's engineering giant, has just received government approval for a merger with its Swiss counterpart, Brown Boveri, and Volvo has bought up Sockerbolaget, Sweden's only sugar company.

But perhaps the most striking merger is that between the companies obliquely referred to in a rhetorical question posed in the recent OECD review of Sweden's national science and technology policy: "Can you imagine today a biochemical laboratory without ultracentrifuges, without electrophoresis, without exclusion chromatography — in short, without Swedish contributions to the science of separation of large molecules, and without Swedish products and equipment adapted to achieve these separations?"

No prizes for guessing that the reference is to Pharmacia and LKB. A small prize if you knew that Pharmacia now

executive, Refaat El-Sayed.

A temporary price, at least, is being paid for Pharmacia's acquisitiveness. Profits were unchanged for the first half of this year despite a massive increase in turnover, and management still has to smooth some of the joins. Most amused by it all are the competitors-turned-colleagues in LKB and the biotechnology division of Pharmacia. The competition had become fiercer over the years as Pharmacia, which began by producing the separation products referred to in the OECD review, and LKB, which began with the separation equipment, each tried to build up their missing halves. The unanswered question is why the complementary halves of the separation business did not get together before now, particularly when they both sprung from the same source, Uppsala University.

It was the university's Theodor Svedberg who both pioneered the use of the ultracentrifuge for protein separation and was a major influence in LKB's foundation in 1943. Many of LKB's products were based on the techniques of electrophoresis devised by Svedberg's student, Arne Tiselius.

And it was Tiselius who persuaded Pharmacia in the late 1950s that it would be worthwhile marketing a cross-linked dextran, Sephadex, for the separation of large molecules by gel filtration. Pharmacia was already selling dextran, discovered by Tiselius's student Björn Ingelman, as a blood plasma volume expander, but had found no use for cross-linked dextrans, until Jerker Porath and Per Flodin, also both students of Tiselius, stumbled upon their value in protein separation. At the time, Flodin was in Pharmacia and Porath in Tiselius's laboratory.

A long-time advocate of the marriage of Pharmacia and LKB, Porath has mixed views on their effect on university research. The individual companies, let alone their combination, are a strong draw for academics, he says, recalling the time that Pharmacia bought out his whole affinity chromatography group.

Nevertheless, he has always been a great advocate of links between science and industry, as makes sense for someone who has continued regularly to contribute new separation techniques to science. In the late 1960s, he and Tiselius formed a company to hold and licence patents arising from their work and so successful has it been that he claims to have paid more back to STU (see below) than any other private individual. The downside, says Porath, who retires from the University of Uppsala this year, was that "15 years ago I was a very suspicious person

even in my own faculty".

Times have changed, and in common with all industrialized countries, there is a new awareness on the part of Swedish scientists of the benefits of flirting with industry. Playing matchmaker is Styrelsen för Teknisk Utveckling (STU), the National Board for Technical Development, which is an agency of the Ministry of Industry.

STU, like its counterparts elsewhere, has a somewhat bewildering variety of



Jerker Porath — one of Arne Tiselius's students and formerly "suspicious" in his faculty.

ways of spending its budget, which amounted to SEK 750 million in 1985/86 (in which year a total of SEK 20,000 million was spent on R&D in Sweden). Its activities range from providing grants for pre-competitive research, in part through a Technical Research Council, to the support of technology and product development. The first of these activities accounts for 45 per cent of STU's budget, and is spent mainly on postgraduate training and major coordinated research programmes, predominantly in computers and electronics. For example, STU was influential in increasing the number of PhDs in microelectronics from 20 in 1978 to 120 a few years later.

Another 40 per cent of STU's budget supports the development and propagation of technology, mostly in cooperative programmes involving both industry and institutes of higher education. The remaining 15 per cent helps pay for product development, and is repayable for any product that is a success. "A 40 per cent success rate was achieved in one of our ten-year programmes", says Göran Friborg, who is involved in long-term planning in the director-general's office at STU, "which is too high. We should be taking greater risks, especially as venture capital is presently withdrawing from high technology".

Information technology, advanced production engineering technology, materials technology, new energy sources and biotechnology are priority areas for STU, but in the last it has suffered a setback. Stating that "what is now needed above all in Sweden is a significant effort in basic research in cell and molecular biology", the government failed to meet STU's request this year for extra funds for biotechnology and instead put them into the higher education coffers. □



Pharmacia Group headquarters in Uppsala.

owns LKB, but a big one if you knew that a major contribution to the group profits of Pharmacia now comes from prescription sales of a chewing gum that substitutes a new form of nicotine intake for the traditional one of smoking.

The gum came with the acquisition of Leo, another Swedish pharmaceutical company — for that is Pharmacia's core business, whatever the perception of laboratory scientists. Leo, LKB and other acquisitions all came in a flurry last year, hard on the heels of an attempted takeover of Pharmacia by Fermenta, which began to go wrong when Pharmacia, among others, questioned the falsely-claimed doctorate of Fermenta's chief

Dependence of creep in olivine on homologous temperature and its implications for flow in the mantle

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Creep data for olivine, when normalized by the melting temperature, can be extrapolated directly to the pressure, temperature and chemical environment of the upper mantle by using the appropriate solidus and geotherms. The data presented here, collected over a broad pressure range, predict effective viscosities that are consistent with geophysical and tectonic constraints for a dry upper mantle. These systematics suggest that the effective viscosity of the mantle has risen over the age of the Earth as outgassing has progressively raised the solidus.

THE nature of convection in the outer 400 km of the Earth's mantle is determined by the rheology of the mineral olivine, $(\text{Mg, Fe})_2\text{SiO}_4$, the most abundant and weakest phase. Experimentally determined creep laws for olivine as a function of temperature, pressure and chemical environment are therefore critical for modelling flow in this region. By measuring the pressure dependence of creep over a larger range of pressures and with an accuracy much greater than previously possible, we have determined that there is a simple relationship between the creep strength of an olivine-dominated rock (peridotite) and melting: at constant homologous temperature (that is, at a constant fraction of the rock's solidus temperature), the strength of peridotite is constant¹. Thus, the effect of pressure and chemical environment on the solidus of a peridotite can be used to quantify the effect of these parameters on creep. Here we combine our homologous temperature relationship for creep of peridotite with previously published melting relationships to extrapolate our data directly to upper-mantle conditions. For a stress of 1.0 MPa, our data predict an effective viscosity of 10^{21} Pa s at 300–400 km, a value consistent with a variety of geophysical constraints. We also conclude that the volatile content of the flowing upper mantle must be low.

The temperature dependence of creep in olivine has been extensively studied at relatively low pressures and high homologous temperatures^{2–6}, and recent work has documented the sensitivity of the creep strength of olivine to changes in the water and oxygen fugacity^{7–11} and silica activity^{11–13}. Previous studies of the pressure dependence of creep have been hampered by the low and narrow range of pressure of most experiments, and by the unreliability of strength measurements in solid-pressure-medium apparatuses⁸. Extrapolations of laboratory data have therefore relied on theories derived from the metallurgical literature^{2,14–18}.

To determine directly the pressure dependence of creep in olivine, we have conducted a series of axial compression experiments at constant strain rate over a pressure range of ~2 GPa, a wider range than has been possible heretofore¹. The experiments were conducted in a modified Griggs apparatus, using a new sample assembly in which specimens enclosed in platinum capsules are surrounded by a molten alkali halide, greatly reducing the friction problems associated with solid-pressure-medium experiments¹⁹. Our experimental material is a synthetic harzburgite (depleted peridotite) composed of 97% olivine and 3% orthopyroxene with a grain size of ~50 μm .

The high-temperature creep of many materials is described by an equation of the form

$$\dot{\epsilon} = A\sigma^n \exp(\Delta Q^*/RT) \quad (1)$$

where $\dot{\epsilon}$ is the strain rate, A is a material parameter, σ is the steady-state flow stress, n is the stress exponent, ΔQ^* is a general activation energy term for creep, R is the gas constant and T

is the absolute temperature. The temperature dependence of creep at constant pressure is generally expressed in terms of an activation enthalpy, and the pressure dependence in terms of an activation volume, where these parameters are assumed to be constant. Over the range of experimental conditions reported here (0.6–2.3 GPa and 1,100–1,500 K), the experimentally determined pressure and temperature dependences of creep are themselves strongly temperature and pressure dependent. As a consequence, extrapolation procedures requiring these parameters become complex and increasingly problematic. Elsewhere we discuss the thermodynamic significance of these results and speculate on their causes^{1,20}.

Alternatively, it has been demonstrated that many material properties, including creep, scale with the melting temperature, leading Weertman^{15,16} to propose that for extrapolation to high pressures equation (1) could be written as

$$\dot{\epsilon} = C\sigma^n \exp(-gT_m/T) \quad (2)$$

where T_m is the melting temperature and C and g are empirical constants. Here, pressure and temperature are replaced by a single parameter, the inverse homologous temperature T_m/T . Our results confirm Weertman's hypothesis.

In Fig. 1, the steady-state flow stress is plotted (on a logarithmic scale) against T_m/T for our experiments. The observed dependence of the flow stress on T_m/T requires that the relationship between the absolute dependences on pressure (P) and temperature be¹

$$\begin{aligned} \left(\frac{\partial \ln \sigma}{\partial P}\right)_{T,\dot{\epsilon}} &= \left(\frac{\partial \ln \sigma}{\partial(T_m/T)}\right)_{\dot{\epsilon}} \left(\frac{\partial(T_m/T)}{\partial P}\right)_T \\ &= \frac{1}{TT_m} \left(\frac{\partial \ln \sigma}{\partial(1/T)}\right)_{P,\dot{\epsilon}} \frac{dT_m}{dP} \end{aligned} \quad (3)$$

This relationship, coupled with equation (1), is equivalent to equation (2) except that the parameter g in equation (2) is not assumed to be a constant. Indeed, the curved solid line (parabola) in Fig. 1a is a better fit to the data than the dashed straight line, indicating that g is not constant.

The dependence of our data only on T_m/T makes extrapolation to the pressures and temperatures of the upper mantle conceptually straightforward. But most creep experiments, including ours, have been performed on more refractory dunites and harzburgites, rather than the chemically undepleted rocks characteristic of the bulk of the olivine-bearing mantle. Important questions arise, therefore, concerning the relationship between the solidus depression caused by the addition of other components and the effect of those components on creep. Our hypothesis corrects for effects on the solidus due to pressure changes. Implicit in the hypothesis, then, is the prediction that changes in the solidus temperature due to composition changes should also be reflected quantitatively in the flow stress, provided

that olivine dominates the rheology. Qualitatively this is true: for example, increasing the water fugacity or silica activity lowers both the solidus and the creep strength⁸⁻¹³. To test this hypothesis quantitatively, we have examined the data of Chopra and Paterson for wet⁹ and for carefully dried⁶ Anita Bay dunite. At the confining pressure of their experiments, 0.3 GPa, the solidus depression of peridotite + H₂O is ~200 K¹⁹. The absolute value of the equilibrium melting temperature of their dunite is unavailable, but fortunately our analysis is sensitive to the water-induced change in solidus temperature, and quite insensitive to the absolute value of the melting temperature. We therefore assume the reasonable melting temperature for dry dunite of 1,900 K at 0.3 GPa and the melting systematics of ref. 22. In Fig. 1b, we collect the Anita Bay dunite data and plot it in the same way as our data in Fig. 1a. Just as normalization to the solidus removes the pressure effect from our peridotite, normalization to the appropriate solidus removes the hydrolytic weakening effect in the dunite.

It appears, therefore, that for an olivine-dominated rheology, changes in the physical conditions or bulk chemistry that change the solidus of a peridotite will have a corresponding effect on the flow stress. This clearly works in the case of changing the pressure or adding water, and we postulate that it should work equally well if Ca, Al, Na and other components of an undepleted peridotite are added. Full resolution of all aspects of these predictions remains to be verified. For example, the strengths of dry Anita Bay dunite and our harzburgite at the same absolute temperature differ by about a factor of three. But at constant homologous temperature, the two data sets are indistinguishable. Of course, in all cases T_m has only been estimated, not measured; the exact agreement of normalized strengths could be fortuitous. We point out, however, that the slopes of the normalized data sets cannot be independently adjusted by our procedure and all three agree within experimental error. Moreover, recent experiments in Paterson's laboratory^{12,13}, conducted on olivine polycrystals in the presence of orthopyroxene and magnetite, yield much lower strengths than ref. 6, comparable to our results extrapolated to 0.3 GPa.

Whether or not the $\ln \sigma$ versus T_m/T relationship is linear is important for extrapolation to mantle conditions; at low homologous temperatures, the slope of the curve is reduced (and hence so are the apparent activation energy and activation volume²⁰). Fitting a linear temperature dependence to data collected only at relatively high homologous temperatures (the common practice) will overestimate the magnitude of this dependence (compare the solid and dashed lines in Fig. 1c). Fortunately, however, our data are collected over a homologous temperature range overlapping the range we estimate below for the upper mantle; extrapolating linearly has no significant effect on our conclusions here, but could have a significant effect if the rheology of lower-mantle silicates is similar and if, as we surmise, the homologous temperatures of the lower mantle are lower.

To extrapolate our data to the upper mantle, we require a solidus for mantle rock, a geotherm for the region of interest and a model of the stress or strain rate with depth. We adopt the dry peridotite solidus derived by Herzberg^{23,24}. This solidus is characterized by a progressively decreasing slope (dT_m/dP) with increasing pressure (Fig. 2a), reflecting the recent discovery that at very high pressures the densities of silicate melts and solids converge (see, for example ref. 25). We have used two representative geotherms: (1) a geotherm for average-aged ocean crust (60 Myr) derived by a simple half-space cooling model²⁶ merged smoothly with a mantle adiabat of 0.5 K km⁻¹, and (2) the 40 mW m⁻² conductive geotherm of Pollack and Chapman²⁷ that passes through the equilibration temperatures and pressures determined from xenoliths in kimberlite²⁸. Both geotherms are further constrained to pass through the olivine-spinel transition at 400 km, requiring a temperature at that depth of 1,725 K²⁹. Using these geotherms and Herzberg's solidus, the bulk of the

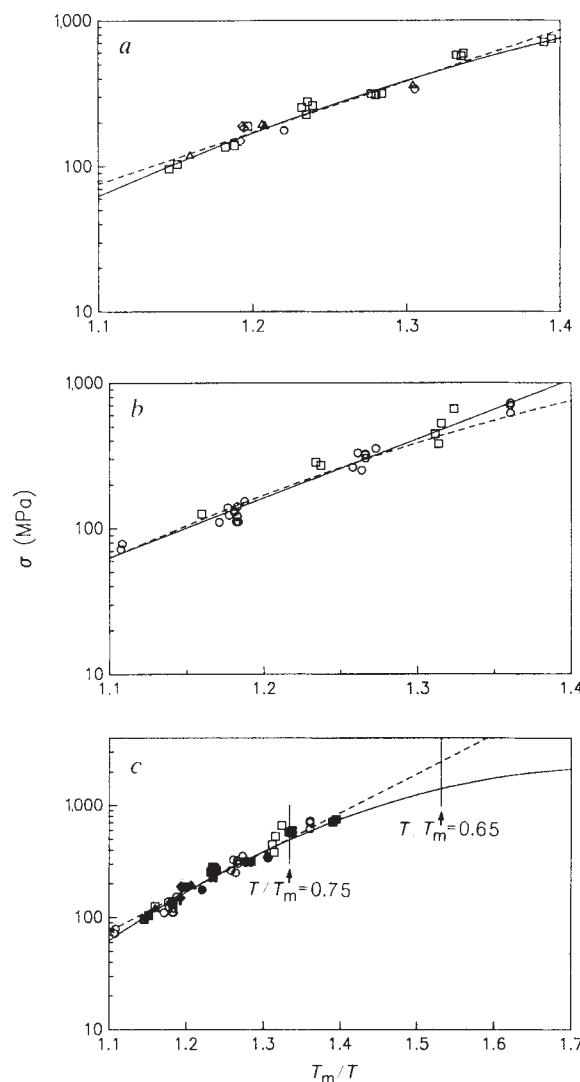


Fig. 1 *a*, Plot of steady-state flow stress against inverse homologous temperature for experiments conducted between confining pressures of 0.6 and 2.1 GPa (mean stresses of 0.7–2.3 GPa), temperatures of 1,100–1,500 K and strain rates of 1×10^{-5} – 3×10^{-4} s⁻¹. Stresses have been corrected to a constant strain rate of 2×10^{-5} s⁻¹ through equation (1) with $n = 3.6$. The slope of the solidus (dT_m/dP) is taken from ref. 40, and T_m at $P = 0.1$ MPa is estimated to be 1,580 K. Experiments conducted at different mean stresses are denoted by triangles (0.7–0.8 GPa), squares (1.1–1.3 GPa), diamonds (1.7–1.8 GPa) and circles (2.2 GPa). For a particular temperature and mean stress, the apparent activation enthalpy ' ΔH^* ' can be calculated by multiplying the slope of the curve by nRT_m and the apparent activation volume ' ΔV^* ' can be obtained by multiplying the slope of the curve by $nRdT_m/dP$; both decrease with decreasing homologous temperature. The solid and dashed lines are, respectively, parabolic and straight-line fits to the data. *b*, The data of Chopra and Paterson^{6,9}, corrected to a constant strain rate of 2×10^{-5} s⁻¹ and plotted as in *a*. T_m^{dry} at $P = 0.1$ MPa is taken to be 1,900 K, the slope of the dry solidus is taken to be 0.08 K MPa⁻¹ and the wet melting systematics are from ref. 22. When plotted against T_m/T , the wet (circles) and dry (squares) data fall along a single curve. The solid line is the linear regression to the data; the dashed curve is the fit to our data in *a*. ' ΔH^* ' can again be obtained by multiplying the slope of the curve by nRT_m ; ' ΔH^* ' calculated in this way for wet dunite is ~90 kJ mol⁻¹ less than that for dry dunite, in agreement with the difference reported by Chopra and Paterson^{6,9}. *c*, Plot of the data of *a* (solid symbols) and *b* (open symbols) on a scale showing the homologous temperature range of the upper mantle (see Fig. 2b). Both the parabolic and straight-line fits from *a* are included. Note that the two extrapolations differ significantly only for $T/T_m < 0.7$.

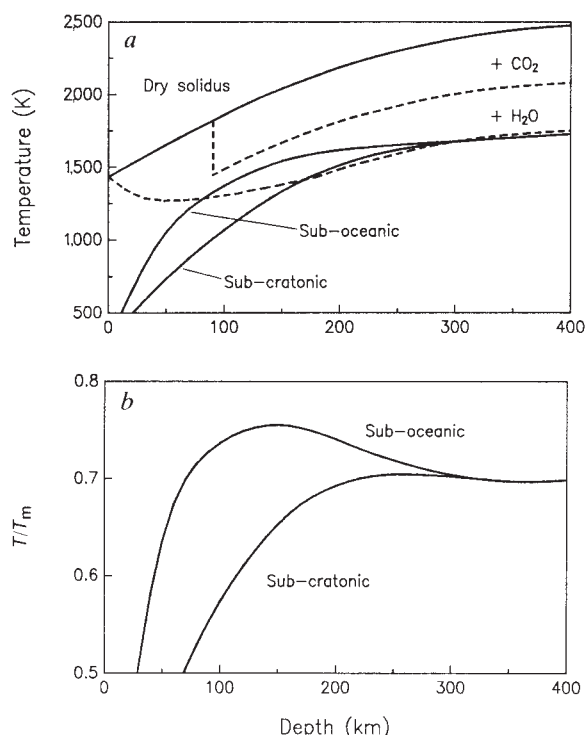


Fig. 2 *a*, The dry peridotite solidus of Herzberg^{23,24} and the CO₂- (ref. 31) and H₂O-saturated²² peridotite solidi. The CO₂-saturated solidus is extrapolated from 3 GPa by assuming parallelism with the dry solidus. The lower curves are representative sub-oceanic and sub-cratonic geotherms. The geotherms are fixed at 400 km by the requirement that the olivine-spinel transition occurs at a temperature of 1,725 K at that depth. *b*, The homologous temperature distribution obtained from *a* for sub-oceanic mantle (upper curve) and sub-cratonic mantle (lower curve). The homologous temperature is mostly between 0.65 and 0.75; these values are indicated on Fig. 1c.

olivine-bearing mantle has a homologous temperature of 0.65–0.75 (Fig. 2b).

Our data have not yet been collected over a sufficient range of strain rates to determine precisely the value of the stress exponent n in equation (1), but our data are consistent with the value of 3.6 obtained by Chopra and Paterson for dry Anita Bay dunite⁹, and other experimental studies of olivine rheology also cluster about values of $n = 3$ –4. We have used $n = 3.6$ to extrapolate our data from the strain rates of our experiments (10^{-4} – 10^{-5} s⁻¹) to those expected in the mantle (10^{-14} – 10^{-16} s⁻¹).

In the mantle we must expect stress and strain rate to be dynamically coupled. However, to avoid obscuring the predictions of our data by model-dependent assumptions regarding this coupling, we take here the simple approach of assuming that the differential stress is constant and equal to 1.0 MPa, a reasonable value for convective processes in the upper mantle^{16,30}.

At the base of the upper mantle, our model with these parameters predicts an effective viscosity of 2×10^{21} Pa s (Fig. 3), corresponding to a strain rate of 0.6×10^{-15} s⁻¹. Although it is difficult to compare effective viscosities with those determined from geophysical measurements by assuming Newtonian behaviour ($n = 1$), these values are of the appropriate order and the predicted strain rate is of the correct magnitude for plate-tectonic motions. For the sub-oceanic mantle, there is a broad low-viscosity channel (asthenosphere) beneath the lithosphere, where the viscosity is about an order of magnitude lower than in the rest of the upper mantle. Below 300 km, the sub-oceanic mantle under a constant stress is essentially isoviscous. For the sub-cratonic mantle, the effective viscosity decreases steadily to

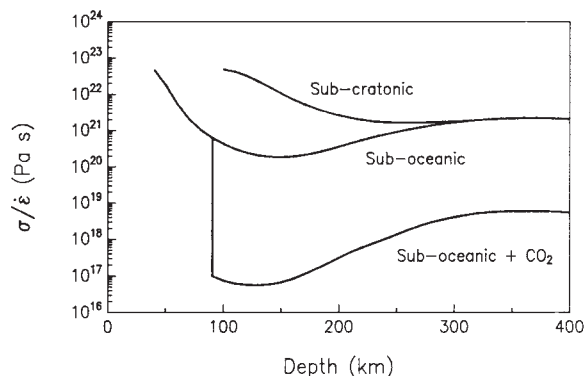


Fig. 3 Effective viscosity mantle profiles for a constant differential stress of 1.0 MPa. For the sub-cratonic mantle (upper curve), the viscosity decreases smoothly to a depth of 200 km; below 200 km the mantle becomes isoviscous. For the mantle beneath 60-Myr-old ocean crust (middle curve), the region immediately beneath the lithosphere is marked by a broad minimum, and below 250 km the mantle is isoviscous. Using the peridotite + CO₂ solidus and the sub-oceanic geotherm (lower curve), a pronounced low-viscosity channel is produced. The effective viscosities predicted are very low.

a depth of 200 km (the base of the lithosphere), below which the mantle becomes isoviscous.

As in all geophysical models, the predictions embodied in Fig. 3 will change if different parameters are used. The most sensitive of these is the assumed stress: changing the level of stress by an order of magnitude produces a change in strain rate of about three orders of magnitude and therefore an effective viscosity ($\sigma/\dot{\epsilon}$) difference of about two orders of magnitude. Changing n by 0.5 produces a corresponding change in effective viscosity of about a factor of 40, and extrapolation of the straight line of Fig. 1c instead of the curve increases the effective viscosity at depths shallower than ~80 km on the oceanic profile and 150 km on the cratonic profile. The straight-line extrapolation creates a more marked discontinuity at the base of the lithosphere in both cases, but has little effect on the sub-lithospheric mantle where flow occurs.

The previous discussion has been limited to a dry peridotite mantle; however, the evidence is overwhelming that volatiles remain in the mantle, with only the abundance and distribution remaining a point of controversy. The effect of volatiles on our model of mantle rheology can be determined through their effect on the solidus, as demonstrated in Fig. 1b for Anita Bay dunite. The dashed lines in Fig. 2a show the CO₂- and H₂O-saturated peridotite solidi. The large drop in the solidus and dramatic weakening of olivine due to water have been addressed above; the sharp drop in the CO₂-saturated solidus at pressures greater than 2.8 GPa³¹ predicts a similar weakening. Experiments are underway in our laboratory to test the weakening hypothesis.

If the upper mantle were water-saturated, a large fraction would be above its solidus—a supposition against which there is abundant evidence. Even for those regions not above the solidus, the homologous temperature would be very high; hence, the effective viscosities would be far too low to be compatible with geophysical evidence. CO₂-induced depression of the solidus implies abrupt and pronounced weakening and therefore a deep low-viscosity channel between depths of 100 and 200 km (Fig. 3). The effective viscosities at 400 km are also too low to be geophysically reasonable. It would appear, therefore, that the upper mantle cannot be saturated throughout with either CO₂ or H₂O (nor any C–O–H fluid of intermediate composition³²). Amounts of either volatile that do not exceed the saturation limit of olivine should produce effects intermediate between the dry and volatile-saturated limits. It is probable,

therefore, that free fluids or hydrous phases in the upper mantle are restricted to cold, shallow levels—that is, to the lithosphere.

A CO₂-enriched (not necessarily saturated) asthenosphere could provide the low-viscosity layer characteristic of many previous models, but the continuous loss of CO₂ at divergent plate margins would require replenishment of CO₂, delivered directly to the asthenosphere. One possible source is from the lower mantle, perhaps via narrow conduits from the core-mantle boundary. These conduits would be plumes of the sort envisaged by Morgan³³ and Loper³⁴, among others, but would derive their low viscosity from their dissolved volatile content (and consequent increase in homologous temperature) as well as their elevated absolute temperature, thus reducing the need to postulate extreme temperature differences across this zone³⁵.

Our results suggest three other important conclusions. (1) Inasmuch as the homologous temperature dependence of creep is not unique to peridotite³⁶, we expect a similar relationship to hold for the mineral assemblage of the lower mantle. Unfortunately, neither the solidus nor the geotherm are well constrained for the lower mantle; hence, quantitative calculations are not possible. Qualitatively, the slopes of both the solidus and the geotherm appear to be low^{29,37}, so we would expect any sig-

nificant effective viscosity contrasts to be confined to boundary layers. (2) Previous evidence from the study of self-diffusion in simpler materials^{17,36,38} has shown that diffusivities, as well as creep strength, track with the melting curve. Our results for creep over a greatly extended pressure range suggest that self-diffusion in the mantle probably follows the same systematics as creep. If so, then any regions of the mantle that may flow by diffusion creep should also follow equation (3), albeit with a different basic rheology. (3) It is almost certain that the original volatile content of the mantle was greater than it is today. Outgassing through time will have resulted in an increase in the solidus (T_m) and a consequent increase in the effective viscosity through equation (2). The tectonic evolution of the Earth may have been controlled in part by whether most outgassing occurred early³⁹, or gradually over a longer time.

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LETTERS TO NATURE

A galactic gravitational lens as the ultimate astronomical telescope

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When a galaxy acts as a gravitational lens it behaves more like a raindrop deflecting sunlight than like a true lens. The images formed by a galaxy exist as directions of incidence of rays on the observer and the best a galaxy can do is to generate complex surfaces called caustics^{1–4}. Here we point out that the caustic surfaces of galactic lenses are powerful telescopes, with point-source intensifications varying with frequency from 10⁵ for radio waves to 10⁸ for X rays. The diffraction limit to the angular resolution varies from 10^{–11} to 10^{–17} arc s from radio waves to X rays and exceeds the resolution of man-made telescopes by many orders of magnitude. The signature of a point source crossing the caustic is a burst of radiation modulated in time by a characteristic

diffraction pattern. The microcaustic surfaces generated by stars in the lensing galaxy are also powerful telescopes that yield large intensifications of small sources crossing the microcaustic. Decaying cosmic strings could be an important source of cosmic rays with energies above 10¹⁰ GeV.

An elliptic lens generates two sets of caustic surfaces in source space⁴. In cross-section far from the lens, the larger of the caustic surfaces has the shape of an ellipse and the smaller surface that of an astroid, with four spikes or cusps. The caustic surfaces delineate the regions where images are created or annihilated. The source must be inside one surface to form three images and inside both surfaces to form five images. The intensity of an extended source on the caustic is limited by surface brightness and has a value of $(y/r)^{1/2}$, where y is of the order of the galactic radius and r the projected source size at the lensing galaxy⁵. For a point source the intensity on the caustic predicted by geometrical optics is infinite. The intensity obtained^{3,5} with wave optics has a value of

$$I \approx \left(\frac{GM}{\lambda c^2} \right)^{1/3} \quad (1)$$

where M is the mass of the galaxy contributing to the lens and λ the wavelength of the radiation at the lensing galaxy. In the

diffraction limit the intensification of a source by the lens is chromatic and varies as $\lambda^{-1/3}$. The intensification is very large and, for $M = 10^{10} M_{\odot}$ (where $M_{\odot}$ is the mass of the Sun), has values of 10^5 , 2×10^6 and 10^8 at wavelengths of 1, 10^{-4} and 10^{-9} cm respectively.

The dimensions of the regions of high gain are important because they determine the expected signature of a point source crossing the caustic surface. For a small distance x along a normal perpendicular to the caustic, the intensity is given by⁶⁻⁸

$$I \propto A_i^2 \left\{ x \left(\frac{8\pi^2}{\rho\lambda^2} \right)^{1/3} \right\} \quad (2)$$

where A_i is the Airy function⁹, and ρ the radius of curvature of the caustic. The intensity falls off exponentially in the shadow region and oscillates with gradually decreasing amplitude in the illuminated region, where I eventually decreases as $x^{-1/2}$. The width of the first Airy fringe is given by

$$\Delta x \approx 2D \left(\frac{GM\lambda^2}{c^2\beta^3} \right)^{1/3} \approx 10^{11} \lambda^{2/3} \quad (3)$$

where D is the distance of the observer from the lens and β the impact parameter of the radiation at the lens. The values of Δx are 10^{11} , 2×10^8 and 10^5 cm at wavelengths of 1, 10^{-4} and 10^{-9} cm, respectively, for a galaxy of mass $M = 10^{10} M_{\odot}$ at redshift $z = 0.5$ (for Hubble constant $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$). A characteristic diffraction pattern will be generated throughout the electromagnetic spectrum for a source with projected size smaller than Δx . The first few fringes of the diffraction pattern will sweep across the observer in ~ 0.03 s in the X ray region, 40 s in the optical region and 10 h at a wavelength of 1 cm, assuming typical galactic motions of 300 km s^{-1} . The detection of the fringe pattern, from a source at a redshift of 2 and a wavelength of 1 cm, yields an angular resolution of 10^{-11} arcs, a value that exceeds by six orders of magnitude that achievable by present radio VLBI (very-long-baseline interferometry) techniques¹⁰. At shorter wavelengths the caustics yield better angular resolution and higher intensifications. The limits on the angular resolution are $\sim 10^{-14}$ and 10^{-17} arcs at optical and X ray frequencies. The value of Δx is small in the X ray region and a neutron star will satisfy the point-source requirement only if the emission originates from a small region above the polar caps. The high gain and angular resolution of galactic caustics make them powerful telescopes for probing structures with small angular size in the distant universe. Furthermore, the galactic lens will intensify neutrino emission and gravitational radiation from the source.

The caustic patterns can be described with the mathematics of catastrophe theory¹¹, in which the Airy caustic is called a fold catastrophe. The fold is associated with the coalescence and annihilation of two images, the third being incidental. More complex caustics arise when two fold regions come together and merge at a cusp and the astroid caustic has four cuspidal regions. The fold catastrophes form surfaces and cusps form lines in three-dimensional space. The intensification of a source on the cusp is given by^{3,11}

$$I \approx \left(\frac{GM}{\lambda c^2} \right)^{1/2} \quad (4)$$

and is typically four orders of magnitude greater at 30 keV than on a fold. The pulsar in the Crab Nebula could be detected at X ray frequencies with about the same intensity if it were at the distance of the quasar 3C273 and situated on a cusp.

The probability of sources at large redshifts being lensed by foreground galaxies has been evaluated¹² and saturates at values between 4×10^{-2} and 8×10^{-3} depending on the cosmological model. But the probability that sources will be on or near galactic caustics is smaller by a large factor because the capture angle for lensing¹³ is much greater than the width of the region of high intensification. To evaluate the probability, the width of a

typical caustic is taken to be equal to that scanned by proper motion of the galaxy, which has a typical value of 10^{-7} arc s in one year. In this case the probability of a background source crossing the caustics is smaller than the lensing probability by an additional factor of $\sim 3 \times 10^{-7}$. The overall probability of a source crossing caustics of intervening galaxies in one year is 1.2×10^{-8} , where a value of 4×10^{-2} has been adopted for the lensing probability. The rate at which quasars or other sources at large redshifts cross the caustics depends on their surface density. Evolutionary models predict¹⁴ 10^7 quasars with absolute blue luminosity less than -23 and redshifts less than 3.5. The large number of quasars combined with the low probability yields a rate of 0.1 quasars per yr crossing galactic caustics.

The intensification of a source crossing the caustic of a gravitational lens depends on its internal structure. The nature of the core regions of quasars and active galactic nuclei are not yet understood. The most plausible models¹⁵ consist of a massive black hole, a superstar such as a spinar or magnetoid and a compact cluster of small-mass black holes and neutron stars. If the nucleus of a quasar is composed of a large number of neutron stars and small-mass black holes, each source crossing the caustic will be intensified by $\sim 10^8$ in the X ray region. The intensification will saturate at a value of 10^3 if the nuclear region consists of a source of uniform surface brightness and size of one light day, as indicated by the light variations¹⁶.

The caustic surfaces are obtained from the smoothed-out mass of the galaxy and ignore the gravitational deflection of individual stars or microlensing¹⁷⁻²¹. Each image produced by the galaxy can be split into a large number of micro-images, but it is usual for a few micro-images with separations of microarc seconds to contain most of the flux. The astroid caustic is formed by rays that pass through the outer regions of the galaxy where the optical depth for microlensing is small and hence the undistorted caustic predicted by wave optics is valid. There will be outbursts at a frequency²² of 10^{-2} yr^{-1} when the source crosses the typical microcaustic of a single star and the time profile of the burst accurately traces the source structure. The elliptic caustic is formed by rays that pass through the galaxy where the optical depth to microlensing is close to unity. The stars give rise to a complex of microcaustics²¹⁻²⁴ in the source and observer planes, and outbursts occur at a frequency of $\sim 0.1 \text{ yr}^{-1}$ as the source crosses the microcaustics. This phenomenon could be an important source of variability in active galactic nuclei²⁴. In the geometrical optics limit²⁵ the intensification of a point source on the caustic is infinite, but for sources of size one light day the gain saturates at 10^2 for solar-mass objects^{22,26}. For smaller sources the intensification increases as $r^{-1/2}$ and has a value of 10^6 at optical and X ray frequencies for a source of size 3×10^7 cm. The damping effect²⁷ on large intensifications is not significant for a small source or cluster or small sources. At radio frequencies a wave optics treatment is required^{28,29} for lenses of less than one solar mass. The microcaustic surfaces are also powerful telescopes for probing the small-scale structure of quasar nuclei.

The probability of a source crossing a caustic surface will be increased if rapid motion is present in the source or lens. In the core regions of some quasars, VLBI techniques have revealed apparent superluminal separation of the radio lobes¹⁰. If compact sources are ejected by quasar nuclei at close to the speed of light and cross the caustic of an intervening gravitational lens, the intensified images cross the Solar System with superluminal speed if the lens is nearer to the quasar than the Earth. The gravitational lensing properties of closed loops of cosmic strings have been analysed and strings give rise to patterns of caustics³⁰ with point-source intensifications comparable to those of a galactic lens. The importance of lensing by loops of cosmic strings is that loops can have high transverse velocities and waves propagating on the string^{31,32} at almost the speed of light. The rapid transverse motion of the string will generate intensified images that cross the Solar System with superluminal speed if

the string is nearer to the source than the Earth. The string loops decay by the emission of gravitational radiation and finally radiate most of their energy in a complex mixture of coherent vacuum-phase waves and high-energy particles with energies up to 10^{15} GeV for grand-unification strings³³. The particles with energy $E > 10^{10}$ GeV will be degraded by interactions with the microwave background for $z > 0.2$. It is a possibility that the highest-energy cosmic rays are the relics of cosmic strings that decayed in intergalactic space at values of $z < 0.2$. The cosmic ray spectrum should extend to energies beyond the limit of the current measurements³⁴ at $E \approx 10^{11}$ GeV to 10^{15} GeV.

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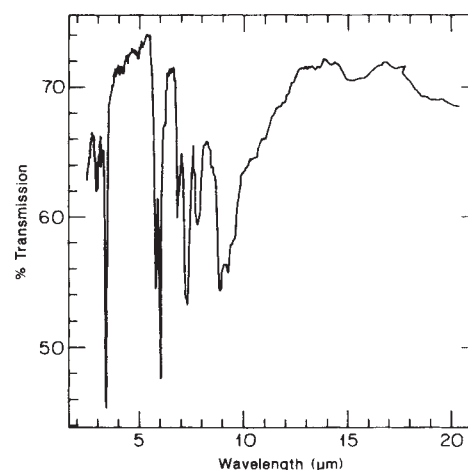


Fig. 1 Transmission spectrum of irradiated methane clathrate residue (after ref. 13).

The 3.4 μm feature has the broad, structureless character expected for a solid-phase origin, although the best resolution available⁴, $\lambda/\Delta\lambda \sim 400$, is insufficient to rule out blending of a variety of lines from gas-phase molecules. If due to infrared fluorescence, a production rate for CH-X molecules in the gas phase ~ 0.2 that for H_2O seems sufficient to account for the observed flux in the 3.4 μm feature in ground-based spectra⁴, in agreement with results from the Vega fly-by (M. Combes, T. Encrenaz & V. I. Moroz, personal communications). Some contribution to the 3.4 μm feature by gaseous emission is therefore expected⁶. However, in this letter we demonstrate that a simple model based on thermal emission from small, hot, organic or organic-coated grains in the Halley coma can readily account for this feature. Such a model is given credence by the fact that Vega mass spectrometry shows that $\sim 80\%$ of the dust encountered in the Halley coma is richer in carbon and nitrogen than C1 carbonaceous chondrites⁷. Further analysis suggests that most particles consist of a predominantly chondritic core surrounded by a complex organic mantle⁸. Thus there is direct evidence for small organic grains in the coma, with typical particle size $\sim 0.1 \mu\text{m}$ (ref. 9).

In cometary ices, clathrate hydrates are thermodynamically favoured¹⁰, and the most probable unprocessed form of hydrocarbons is the methane clathrate. Proton¹¹ or γ -ray¹² irradiation of pure CH_4 ice produces an organic residue that shows a 3.4 μm absorption feature as does charged particle irradiation of CH_4 clathrate and other hydrocarbon/ H_2O ices^{13,14}. The transmission spectrum for one such irradiated ice residue, that of a low-occupancy (200:1) $\text{H}_2\text{O}:\text{CH}_4$ methane ice clathrate, is shown in Fig. 1. (This feature is by no means present in all organic solids; it is absent in the solid irradiation products of simulated uranian and neptunian atmospheres¹⁵.) As comet Halley approaches the Sun, icy grains are ejected from its surface or jetted from its interior, and the ice sublimates. The refractory organic residue, probably stable to temperatures of 500–1,000 K (ref. 16), remains largely in solid form (though some evaporation may contribute to gas phase fluorescence) and, heated by solar radiation, emits in the infrared. This process is well modelled in our laboratory experiments, where the ice is first irradiated, then completely evaporated by high-vacuum pumping at temperatures ~ 425 K before the transmission spectrum of the residue is determined¹⁴.

A number of regimes can be distinguished for the irradiation of C-rich ices in comet Halley. We have systematically examined this irradiation history elsewhere¹⁷. In its present short period orbit, Halley experiences a flux of ~ 1 keV solar wind particles, with a typical penetration depth in ice $\leq 1 \mu\text{m}$. During its ~ 4.5 Gyr residence in the Oort clud, the nucleus experienced a total cosmic ray dose $\sim 10^{21}$ keV cm^{-2} of ~ 1 GeV particles, with

Infrared emission by organic grains in the coma of comet Halley

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Spacecraft¹ and ground-based^{2–5} observations of comet Halley in the near-infrared reveal a triple-peaked emission feature near 3.4 μm , characteristic of C–H stretching in hydrocarbons ($-\text{CH}_3$ and $-\text{CH}_2-$ alkanes). Here we discuss transmission spectra of organic residues produced by laboratory irradiation of candidate cometary ices, which serve as the basis for a model of emitting cometary dust. The laboratory synthesis of solid organic residue from irradiated low-occupancy methane ice clathrate simulates the radiation processing experienced by comet Halley. The transmission spectrum of this residue, convolved with a simple two-component thermal emission model based on the spacecraft-determined dust distribution in the Halley coma, fits the 3.4 μm feature, provides optical depths in excellent agreement with those observationally determined, and accounts for the absence of features at longer wavelengths (despite their presence in transmission spectra of typical ice irradiation residues).

significant irradiation extending to depths of 10 m or more. Finally, during its first $\sim 10^6$ – 10^7 yr the entire nucleus was processed by decaying radionuclides (chiefly ^{26}Al) incorporated into the comet during its formation, perhaps responsible for a dose $\sim 10^{20}$ keV cm^{-3} (ref. 18). Integration of the observed production rates of OH and dust suggests that comet Halley loses a depth ~ 5 m each orbit, so that most of the irradiation products due to solar wind and cosmic rays should be quickly lost to space. The observed low-albedo crust of the comet may be of primordial origin¹⁹. While some fraction of the emitting dust may be fragments of this crust, most is probably jetted from the interior, with organics thus due mainly to processing by radionuclides and pre-accretion irradiation.

Laboratory irradiation of CH_4 clathrate¹⁴, and other C-containing ices^{20,21} indicates a synthesis efficiency of 1–10 C or O atoms incorporated into involatile organic product per keV applied to the sample. The total yield of *all* products (including more volatile simple hydrocarbons through C_5 and probably CO), is higher by about an order of magnitude¹⁴. Were CH_4 present in saturated clathrate form ($\text{CH}_4 \cdot 5.75\text{H}_2\text{O}$), for an average cometary density ~ 1 g cm^{-3} there would be $\sim 5 \times 10^{21}$ C atoms cm^{-3} . This is almost certainly a considerable overestimate of the amount of CH_4 present²². Typically $[\text{C}]/[\text{O}] \geq 1$ for such organic products. Thus we have the conservative conclusion that the succession of radiation environments experienced by comet Halley before being perturbed into its present short-period orbit should result in an outer cosmic ray-processed layer 1–10 m thick, irradiated at $\sim 10^{21}$ keV cm^{-3} , in which $\sim 100\%$ of all CH_4 has been converted to (volatile and involatile) organic products, overlying an interior uniformly processed by radionuclides at a dose $\sim 10^{20}$ keV cm^{-3} , in which $\sim 10\%$ of the CH_4 inventory has been processed.

Figure 2 shows the spectrum of comet Halley observed by Wickramasinghe and Allen² on 31 March 1986 (heliocentric distance $R = 1.16$ AU, geocentric distance $\Delta = 0.549$ AU), and our best fit (see below) to their data. The overall shape of the spectrum is that of a continuum which is well-fit by the sum of scattered sunlight (known from observations made that night at shorter wavelengths) and a 350 ± 10 K black body. The emission feature at $3.4 \mu\text{m}$ is evident. In addition, there is an absorption feature between 3.0 and $3.1 \mu\text{m}$, which is apparent in other ground-based³ and spacecraft¹ observations; it has been tentatively identified as due to H_2O ice¹. We have not incorporated such absorption into our model.

At a heliocentric distance $R = 1.16$ AU, a black body would be expected to have a temperature ~ 280 K. The fact that the shape of the continuum is best fit for 350 ± 10 K indicates the presence of small (submicrometre) grains which, being inefficient emitters in the infrared, heat to temperatures well above black body equilibrium. In reality, of course, no single temperature can be ascribed to the dust responsible for the continuum emission: the observed flux is an integral over emission from grains of all sizes, each at different temperatures. In the optically thin case (an assumption justified below), we have

$$F_\lambda = \Omega \int \tau(\lambda, a) B_\lambda[T(a)] da \quad (1)$$

where $B_\lambda[T(a)]$ is the Planck function per μm wavelength per particle radius a , Ω is the solid angle subtended by the telescope at the source, and the wavelength- and radius-dependent optical depth $\tau(\lambda, a)$ is given by

$$\tau(\lambda, a) = \int n(a) \pi a^2 Q(a) dl \quad (2)$$

Here $n(a)$ is the number density, πa^2 is the geometrical cross section, and $Q(a)$ is the extinction efficiency factor for a particle of radius a . The integral is taken along the line of sight through the source.

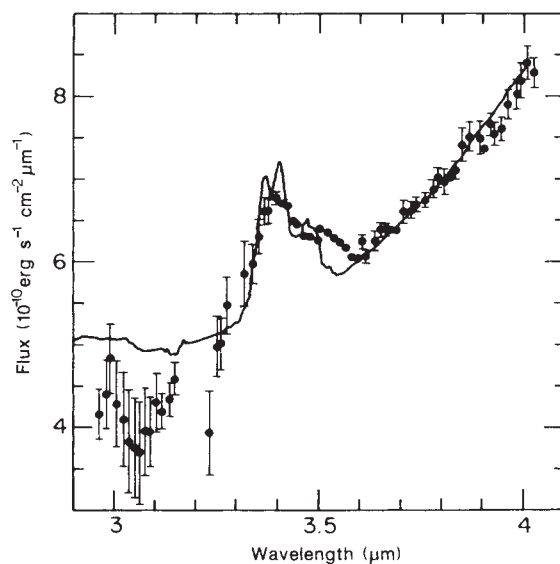


Fig. 2 Best fit (solid curve) to 3–4 μm spectrum of comet Halley (ref. 2). The absorption feature at $\sim 3.1 \mu\text{m}$, probably O–H stretch, is not modelled.

Modelling dust emission from an actual comet is a matter of making sufficient approximations to allow equations (1) and (2) to be computed. $Q(a)$ is not only a function of particle radius, but of chemical composition and frequency as well; $T(a)$ depends on the absorptivity of the grain near the peak of the solar spectrum; and $n(a)$ is in general unknown.

Hanner²³ has fit cometary continuum emission with a two-parameter model for $n(a)$, calculating $T(a)$ via Mie theory. For $\lambda \gg a$, $Q(a) \propto 2\pi a/\lambda$, with the proportionality factor composition-dependent²⁴. The Giotto spacecraft measured the mass spectrum of dust along its trajectory through the Halley coma²⁵. Using the Divine *et al.*²⁶ pre-encounter model of the density-radius relationship, this mass spectrum yields either the geometrical filling factor (cross section per unit area) or number density of dust along the Giotto trajectory as a function of particle radius²⁵. Unfortunately, we cannot hope to use the resulting distribution in more than a crude way to model infrared emission from comet Halley, due to its extreme time-variability. Most comets exhibit a $1/R^4$ brightness dependence in heliocentric distance R , indicating a $1/R^2$ dependence for dust production. This general trend is observed for comet Halley as well, although the comet varies by as much as a factor of 7 from this trend over several days²⁷. Indeed, over a period of just 3 nights' observations, Wickramasinghe and Allen found a 4-fold variation in overall flux, and an uncorrelated variation of a factor of 4.5 in the emission feature. Similarly, 5–13 μm observations show that the 10 μm silicate dust feature varies by as much as a factor of 2 relative to the continuum²⁸. Thus the composition and/or $n(a)$ of the emitting dust is highly time-variable. There are two weeks between the Giotto and Vega fly-bys and the Wickramasinghe and Allen observations. To evaluate equations (1) and (2) for the ground-based spectra therefore requires the introduction of free parameters to describe the dust distribution, or else a much simpler model. In this letter, we use a simple two-component model to explain the spectrum shown in Fig. 2.

The simplest model for dust emission from the Halley nucleus—constant velocity outflow for particles of a given radius—predicts that dust number density $n(a, r)$ should vary as $1/r^2$, where r is the nuclear distance. This model is roughly confirmed by spacecraft observations²⁵. The trajectory of the Giotto spacecraft defines a chord through the Halley coma, with closest approach to the nucleus $r_G = 600$ km. With the $1/r^2$ relation, the geometrical filling factor $A_G(a)$ integrated along

the Giotto trajectory for particles of radius a can be determined:

$$A_G(a) = 2 \int_{r_G}^{R_c} n(a, r) \pi a^2 dr \approx 2n(a, r_G) r_G \pi a^2 \quad (3)$$

where $R_c \equiv 5 \times 10^4$ km is the radius of the coma. Combining the Divine *et al.*²⁶ density-radius model for the Halley dust with the empirical formula for dust fluence as a function of mass experienced by Giotto²⁵, we find the total filling factor summed over all particles to be $A_G = 8.3 \times 10^{-4}$.

Individual dust grains with $a \gg \lambda$ are optically thick at wavelength λ , and thus contribute an optical depth to emission at that wavelength (at which they emit as black bodies) appropriate for their geometrical cross section. Taking particles with $a \geq 10 \mu\text{m}$ to be optically thick at the wavelengths of interest here, we find such particles comprise $\sim 77\%$ of the geometric cross section in the Halley coma.

Wickramasinghe and Allen observed using a rectangular aperture measuring 10×5 arc seconds, centred on the position of greatest infrared flux. Taking this as the nucleus, we can integrate A_G , correcting for its variation with $1/r^2$, to find the total geometric cross section, A_{tot} of all dust in their field of view. We find $A_{\text{tot}} \propto$ aperture size; that is, we recover the usual result that observed thermal flux is proportional to aperture size²⁷. Incorporating the $1/R^2$ dependence of dust production on heliocentric distance (for 14 March 1986, the day of the Giotto fly-by, $\Delta = 0.96$ AU and $R = 0.90$ AU), integration gives $A_{\text{tot}} = 1.4 \times 10^3 \text{ km}^2$, which is the total geometric emitting area of the dust in the Wickramasinghe and Allen aperture. With a radius ~ 5 km, the nucleus contributes $\sim 80 \text{ km}^2$, or about 5% of the total emitting area. Thus the observed continuum radiation is due almost entirely to the dust in the coma. Yet this dust is optically thin: dividing A_{tot} by $\Omega \Delta^2$, we find a geometrical filling factor $A_{\text{WA}} = 1.9 \times 10^{-4}$.

The simplest approximation to equation (1) for the continuum radiation is to treat $\tau(\lambda, a) = A_{\text{WA}}$, and the Planck function to be that appropriate for $T = 350$ K. Such an approximation crudely averages together the effects of the small, hot particles with the larger, optically thick particles at 280 K. This approximation mitigates the need to 'fine tune' several parameters, and we will demonstrate that it works extremely well.

In our model, the emission feature at $3.4 \mu\text{m}$ is due to small, hot, optically thin organic or organic-coated grains. The transmission spectrum of the organic residue of irradiated CH_4 clathrate allows us to model the wavelength-dependent optical depth of such grains directly. We have $\tau_\lambda = N_1 \sigma_\lambda$, where N_1 is the column density of the residue in the coma and $\sigma_\lambda = N_2^{-1} \ln(\tau_\lambda^{-1})$ is the wavelength-dependent cross section derived from the laboratory-measured transmission. Here N_2 is the column density through the residue sample in the laboratory, and t_λ is the transmissivity of the sample (given by Fig. 1, with maximum transmissivity set equal to 1).

Using the optical constants²⁹ for an organic residue formed by sparking 10% CH_4 in a nitrogen atmosphere, both Hanner³⁰ and Lamy and Perrin³¹ have found that $0.1 \mu\text{m}$ grains will reach temperatures ~ 500 K at ~ 1 AU; $0.1 \mu\text{m}$ grains composed of magnetite or glassy carbon will have somewhat higher temperatures (~ 600 K) at this heliocentric distance²³. As the optical constants of irradiated ice residues are at present unknown, we adopt ~ 500 K as a temperature for the emitting organic grains. We therefore derive the following expression for the $3\text{--}4 \mu\text{m}$ flux measured by Wickramasinghe and Allen: $F_\lambda = C_\lambda + \Omega \tau'_\lambda B_\lambda(T = 500 \text{ K}) + \Omega \tau B_\lambda(T = 350 \text{ K})$, where C_λ is the scattered solar flux and $\tau'_\lambda = \beta(a/\lambda) \ln(\tau_\lambda^{-1})$. Here β and τ are parameters varied to provide a best χ^2 fit to the data. In practice τ is tightly constrained by the Giotto-determined filling factor in the Halley coma, and an upper bound is placed on τ'_λ (hence β) by the filling factor represented by the optically thin grains.

Our best fit is shown in Fig. 2; χ^2 -minimization yields the values $\tau = 1.4 \times 10^{-4}$, and $\beta = 1.1 \times 10^{-5}$ (giving $\tau'_\lambda = 1.1 \times 10^{-7}$

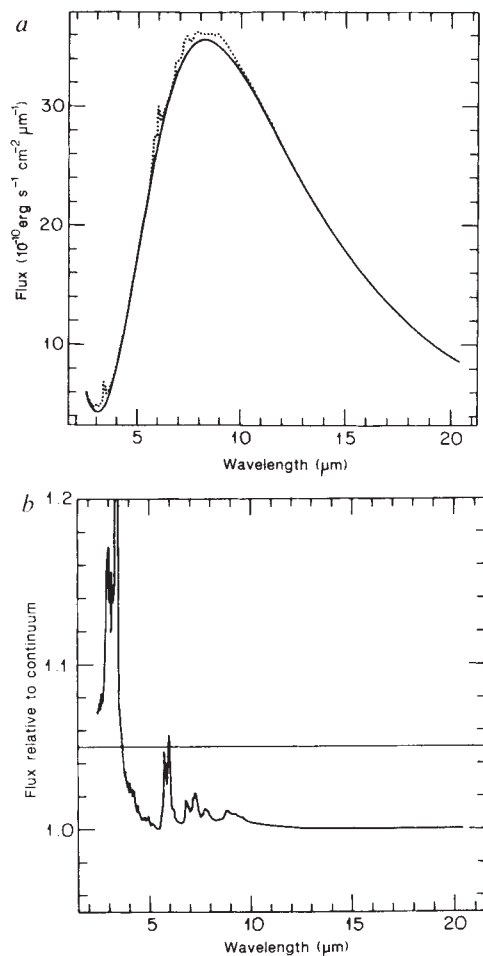


Fig. 3 *a*, The comet Halley 2–20 μm spectrum predicted by our model (taking $T \sim 500$ K for the small organic emitters), compared to that of a 350 K blackbody. *b*, The predicted 2–20 μm spectrum, after dividing out the continuum. Only the $3.4 \mu\text{m}$ feature is resolvable above the 6%-above-continuum level (indicated by the solid line).

at $\lambda = 3.4 \mu\text{m}$). τ compares very well with the value predicted by a simple geometric filling factor model: τ is within 25% of the value A_{WA} found by integrating the Giotto-determined dust distribution (adjusted for heliocentric distance) over the aperture used by Wickramasinghe and Allen. This agreement is much closer than the highly variable nature of Halley dust emission allows us to expect.

The optical depth found for the small organic grains $\tau'_\lambda = 8.0 \times 10^{-4} \tau$, lying well within the upper bounds placed by the Giotto-determined dust distribution. Combining the Divine *et al.*²⁶ density-radius relationship with the Giotto mass spectrum²⁵, we find that dust particles with $a \leq 0.1 \mu\text{m}$ contribute a filling factor $\sim 1.0 \times 10^{-3} A_G$; particles $\leq 1.0 \mu\text{m}$ contribute $\sim 2.2 \times 10^{-2} A_G$, and particles $\leq 10 \mu\text{m}$ $\sim 0.23 A_G$, where A_G is the total filling factor found by Giotto.

The triple-peaked emission feature predicted by the laboratory-measured transmission spectrum is seen to be slightly too narrow to match perfectly the observed Halley spectrum. The width of the laboratory feature is, however, somewhat influenced by radiation dose and temperature, dependencies which we intend to explore in future experimental work. Finally, we must address the question of other emission features predicted by our model. The transmission spectrum in Fig. 1 exhibits a variety of features at $\lambda > 3.4 \mu\text{m}$, not present in a set of 5–13- μm spectra of Halley's comet taken by Bregman *et al.*²⁸. However, if the parameters determined above in modelling the 3–4 μm spectrum are used to model the Halley spectrum out to 20 μm (Fig. 3*a*),

it is found that all higher-wavelength features evident in Fig. 1 are greatly suppressed relative to the continuum. The continuum optical depth $\tau \sim 10^3$ times the optical depth due to the emitting organics (τ'_λ), so that only near the minimum in the continuum radiation can an organic emission feature make a significant contribution. The 3.4- μm feature is the sole feature near that minimum, lying at the intersection of the scattered solar and 350 K black body curves.

In Fig. 3b we divide out the continuum to examine the strength of the individual emission features. Only the 3.4 μm feature rises over the level 10% above continuum, and the peaks at $\sim 6 \mu\text{m}$ are too narrow to be detectable at the wavelength resolution ($\Delta\lambda/\lambda \sim 0.02$) of the Bregman *et al.*²⁸ observations above the ~ 5 –6% over background level. Although these observations show no 6 μm feature to the 2% level (L. Allamandola, personal communication), we consider the predictions of our model to be in sufficient agreement with this limit of detectability, as the optical depths appropriate for fitting the 3.4 μm feature for 31 March 1986 cannot be exactly appropriate for other dates (indeed, had we fitted Wickramasinghe and Allen's observations of 30 March, the predicted 6 μm feature would be resolvable only below $\sim 3\%$ above continuum). Among other uncertainties, the strength of the 6 μm feature relative to that at 3.4 μm is expected to vary with radiation dose; CH_4 clathrate is certainly not the only C or N-containing ice initially present in comet Halley; the temperature 500 K calculated from optical constants for a gas-phase N-rich product may not be correct for an ice residue [indeed, taking $T \sim 600$ K for the organic emitters (the temperature appropriate for glassy carbon grains) reduces the strength of the 6 μm feature by a factor of 2 (ref. 17)]; and most importantly, the simple two-component model used here is too crude to expect precise agreement with observation. Nevertheless, even this simple model demonstrates a physical mechanism for the suppression of higher-wavelength features relative to that at 3.4 μm .

As the comet moves away from perihelion, the intersection of the scattered solar spectrum and the comet's thermal emission spectrum will move to longer wavelengths. Thus, relative to the continuum, we expect the 3.4 μm feature to be suppressed and those at longer wavelengths progressively enhanced—as long as the comet retains a coma. Future comet rendezvous missions will be well placed to test this prediction of our model (which we present quantitatively elsewhere).

Finally, we note that the continuum temperature in the 5–13 μm range²⁸, adjusted for heliocentric distance, agrees to within the uncertainty with the 350 ± 10 K continuum temperature found by Wickramasinghe and Allen. That is, approximating continuum emission by a 350 K temperature averaged over all dust sizes remains valid through the wavelength region of interest. Herter *et al.*³² have shown, however, that the continuum emission cannot be fit by a single black body temperature to 30 μm , where the more rapid fall-off of high-temperature emission by small grains evidently begins to render our approximation invalid.

We thank W. Reid Thompson for helpful discussions, as well as Louis Allamandola, Steven Beckwith, Michel Combes, Andrew Clegg, Thérèse Encrenaz, Martha Hanner, Paul Helfenstein, Bishun Khare, Vasily Moroz, and Tony Phillips; and are especially grateful to Drs. Khare and Thompson for permission to reproduce Fig. 1 from ref. 13. This research was supported by NASA.

Note added in proof: Allen and Wickramasinghe³³ have now reported observations of a 3.4 μm emission band in comet Wilson, closely matching the comet Halley feature for approximately the same heliocentric distance. It is surprising that two comets with such evidently different histories should display such nearly identical infrared spectra: Apparently the characteristics of cometary organics are remarkably similar for both dynamically new and old comets strengthening the case made here for the primordial origin of these molecules.

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Atmospheric radioactivity and variations in the solar neutrino flux

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Measurements¹ of the flux of neutrinos emitted as by-products of hydrogen fusion reactions in the Sun's core bear on questions ranging from stellar evolution theories^{2,3} to the question of the neutrino mass. Attention has been devoted recently to possible time variations in the neutrino flux data, and some investigators have found an inverse correlation with the 11-year sunspot cycle^{4–7}, although there is still some debate about the statistical significance of this correlation⁸. The neutrino luminosity of the Sun is not expected to vary on timescales less than one million years, so alternative sources of neutrinos have been invoked to explain the temporal variations. Galactic cosmic rays have been considered to be a likely candidate, as their intensity at the Earth's orbit varies inversely with the activity of the Sun. Here we present a calculation of the inventory of positron emitters in the Earth's atmosphere that results from hadronic cascades initiated by galactic cosmic rays, and the variation of this source of low-energy neutrinos with the 11-year solar cycle. Our results rule out the possibility that cosmogenic neutrino emitters are responsible for an apparent solar-cycle dependence in Davis's solar neutrino experiment¹. As atmospheric secondary-particle decay was previously eliminated as a significant neutrino source⁹, any such variations, if corroborated over the next sunspot cycle, would appear to be caused by phenomena outside the Earth's atmosphere, most likely in the Sun itself.

Table 1 Dependence of neutron monitor counting rate, star production rate in the atmosphere, atmospheric radioactivity, and neutrino flux on solar activity

Solar activity level	Neutron monitor counting rate (counts h ⁻¹)	Average star production rate (cm ⁻² s ⁻¹)	Total activity (MCi)	Neutrino flux (cm ⁻² s ⁻¹)
Minimum	2.15 × 10 ⁶	1.94	13.14	9.53 × 10 ⁻²
Mean	2.05 × 10 ⁶	1.83	12.43	9.02 × 10 ⁻²
Maximum	1.77 × 10 ⁶	1.40	9.47	6.87 × 10 ⁻²

Hourly counting rates of ground-level neutron monitors at Deep River, Canada, were used to characterize solar modulation. The star production rate shown corresponds to an average column of the atmosphere 1 cm² in cross-section. The total activity (MCi) is from neutrino emitters decaying in the whole atmosphere and the ensuing neutrino fluxes correspond to any point inside the thin atmospheric shell. The atmosphere was taken as a spherical shell 40-km thick with an inner radius of 6,371 km, having an exponential density with a scale height of 6.2 km.

Cosmic-ray interactions in the atmosphere yield secondary particles, which decay in flight, producing high-energy neutrinos. Their contribution to the event rate reported by Davis and co-workers¹ is about three orders of magnitude below the observed value⁹. A concurrent mechanism producing neutrinos is the spontaneous decay of radioactive fragments of spallation of atmospheric constituents by secondary hadrons.

To quantify the neutrino flux from radioactive decay in the atmosphere, we used the methodology described by O'Brien¹⁰ and O'Brien and de la Zerda¹¹. To study time-dependent cosmic-ray phenomena, the primary cosmic-ray spectra must be known as a function of time. This is achieved by using results of O'Brien and de P. Burke¹² relating primary cosmic-ray spectra to the counting rates of ground-level neutron monitors. At any given instant in the solar cycle, we used the primary spectrum corresponding to the counting rate observed at that time, and evaluated secondary proton, pion and neutron spectra by solving the stationary form of the Boltzman equation by means of a straight-ahead transport code¹³. The production of high-energy inelastic collisions in the air (also known as nuclear 'stars' from the days of photographic plate studies) is then calculated.

The star density (in stars per gram of air per second) was evaluated as a function of atmospheric depth and geomagnetic latitude. After integration, we obtained the average production rate of stars in a column of air of 1 cm² cross-sectional area (see Table 1). The 'yield per star' of a given isotope is obtained by multiplying the secondary hadron energy-spectra by the corresponding spallation cross-sections¹⁴ and integrating the result over energy. Yields of 40 positron emitters (which are therefore neutrino emitters) from spallation of nitrogen, oxygen and argon were calculated assuming average hadron spectra; that is, those obtained at 45° geomagnetic latitude, at an altitude of 10 km and for mean solar activity. The most abundantly-produced neutrino emitters are ¹³N, ¹¹C and ¹⁵O, about one nucleus of each isotope being produced for every 100 stars.

From the star production rates and the isotopic yields, production rates of the isotopes of interest were obtained. Equilibrium between production and decay was assumed, so that the rate of neutrino emission is identical to that of radioisotope production. The total radioactivity in the atmosphere results when we sum over the Earth's surface and add the contributions from all isotopes. Table 1 also shows the total radioactivity and the ensuing flux of neutrinos.

The mean neutrino flux from cosmogenic radioactivity in the atmosphere is thus calculated to be 0.090 cm⁻² s⁻¹, while the amplitude of the variation between solar minimum and maximum is only 0.027 cm⁻² s⁻¹. These neutrinos have energies of between 1 and 10 MeV and contribute on the order of 10⁻⁹

SNU to the capture rate in the chlorine detector. The combined uncertainty from utilization of Rudstam's spallation cross-sections and from our assumption of a uniform source of neutrinos is estimated to be less than a factor of three. Thus, the contribution to the neutrino capture rate from cosmogenic isotope decay is six orders of magnitude smaller than that from the neutrinos from secondary particle decay, and nearly nine orders of magnitude smaller than the event rate observed in the chlorine solar neutrino detector.

The apparent negative correlation between neutrino capture rate and solar activity cycle can not be accounted for via cosmic-ray activation of the atmosphere. This finding, together with the results of Gaisser and Stanev⁹ which showed that the contribution from atmospheric secondary particle decay is also insignificant, have an important implication, namely, if such variations are real, their cause is most likely to derive from the Sun itself.

At present, resonant oscillation of neutrino 'flavour' appears as a plausible explanation for the lower-than-expected solar neutrino flux¹⁵. However, if the ³⁷Cl experiment data over the next solar cycle corroborate the dependence on solar activity, we will be confronted with the second solar neutrino riddle.

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Volcanic input to the atmosphere from Alba Patera on Mars

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With the recent increased interest in the evolution of volcanism, tectonism and volatiles on Mars, several studies have been initiated to infer the mass of water vapour and carbon dioxide that might have been released from magmas erupted on the martian surface over geological time (see refs 1, 2). Such studies are complementary to investigations of the global volatile inventory for Mars,^{3,4} which trace a constant mass of volatiles to various sinks in the regolith, atmosphere and polar caps. Of necessity, these volatile distribution models are generalizations of Mars-wide averages. Here we present

Fig. 1 Generalized map of Alba Patera (modified from ref. 5), showing the area from 32.5° N to 47.5° N and 100.0° W to 122.5° W. The two summit calderas are indicated by arrows. The distribution of graben that almost enclose Alba Patera are outlined. Shaded areas are the locations where channel networks exist on the flanks of the volcano. Large meteorite craters (barbed circles) and areas where cloud cover or poor image resolution (dashed lines) preclude identification of certain features are also indicated.



what we believe to be the first estimates for the amount of water vapour and/or carbon dioxide released, together with the corresponding release rates, from specific volcanic deposits on a relatively young martian volcanic construct (Alba Patera).

Recent morphological studies⁵ of the volcano Alba Patera (40° N, 109° W) have indicated that large areas of the lower flanks of the volcano are likely to be covered by pyroclastic deposits. These materials were recognized by the observation that numerous channels of apparent non-volcanic origin are located on the volcano at radial distances of 200–450 km from the summit calderas (Fig. 1). Analysis of 10–50 m per pixel Viking Orbiter images, and the resultant map of the distribution of the channels across the flanks of Alba Patera, suggest that the channels originated by sapping of groundwater from within the unconsolidated materials on the flanks⁵. Supporting diagnostic evidence for the origin of these channels includes their characteristic digitate pattern and amphitheatre headwalls, which are usually attributed to sapping^{6–8}, and the lack of lava flow lobes or other morphological features that are seen on young martian lava flows at this resolution^{9,10}. Elsewhere⁵, we argue that the occurrence of these sapping channels and the absence of lava flows within the channel area on Alba Patera indicate that the flank materials are most probably unconsolidated, analogous to the deposits on the martian volcano Hecates Tholus. In addition, Viking Orbiter infrared thermal mapper (IRTM) data indicate a very small (3–10 μm) mean particle size for these surface deposits on Alba Patera, which is inconsistent with consolidated lava flows¹¹. We favour a pyroclastic origin for these deposits that surround the summit⁵.

Based on numerical models of explosive volcanic eruptions in the martian environment^{12,13}, these materials are most likely to be pyroclastic flow deposits generated by ignimbrite-forming eruptions of Alba Patera early in its evolution. After their emplacement, the flow deposits were invaded by volatiles (probably including both carbon dioxide and water) from the atmosphere. The eruption of summit and sub-terminal lava flows subsequent to this volatile-charging caused the release of volatiles as hot lava flows overrode the volatile-rich surface on the northern (topographically lower) flanks, with the result that an extensive channel system of fluvial origin was produced. We deal here not with the later, atmospheric volatiles which invaded the pyroclastic flow materials but with the magmatic volatiles released in the eruptions that emplaced the pyroclastic flows. We explore the implications of the ignimbrite-forming style of activity on Alba Patera, not only for the necessary volatile content of the erupted magma, but also for the most likely source of these volatiles and their subsequent impact on the martian climate.

The unique morphology of Alba Patera was first recognized in Mariner 9 images of the volcano, and subsequent studies

using Viking Orbiter images revealed a low-relief shield volcano >1,000 km in diameter^{14,15}, that rises ~3–4 km above the plains to the east and 2 km above the region to the south¹⁶. At the summit of Alba Patera there are two summit calderas, one complete and the other partially infilled by later lava flows. The complete caldera is 65 × 45 km in diameter and 0.15 km deep (giving a volume of 345 km³), while the partially buried caldera is ~80 × 50 km in diameter and 200 m deep (volume = 628 km³)¹⁵. These calderas are not single collapse features; rather they resemble other martian shield volcanoes¹⁷ in having experienced multiple collapse events that typically produced nested calderas each of which is ~25 km in diameter. At least six nested calderas can be recognized at Alba Patera, and we estimate the typical volume of a single collapse event to be ~60 km³. There are inevitably assumptions that have to be made regarding the degree of preservation of the complete collapse of the magma chamber/caldera, and the relative volume of the ignimbrite that was erupted compared to the more recent eruptions of lava. Here we take this volume of 60 km³ for the caldera collapse events to be representative of the minimum magma chamber volume for Alba Patera, in accordance with the arguments of Wood¹⁸.

From recent stereo photogrammetric measurements of the volcano¹⁶ and the lateral extent of channel deposits (Fig. 1), we estimate that the average height of Alba Patera above the surrounding plains is ~3 km. Thus, the total volume of the volcanic deposits approximates to: $1/3 \times \pi \times 460^2 \times 3 = 664,500 \text{ km}^3$. Using a typical density of 2,000 kg m⁻³ for such volcanic materials, this volume is equivalent to a dense rock (2,600 g m⁻³) volume of ~510,000 km³. If Alba Patera had a mean magma supply rate that was typical of the long-term (>1 Myr) activity of terrestrial volcanoes (we assume a range of magma output rates from 10⁻² to 10⁻³ km³ yr⁻¹; ref. 19), then the time taken for this volume of material to have been erupted would be ~50 million–0.5 billion years. (Note that, on timescales of this magnitude, the repose period between individual eruptions becomes unimportant, as it is the long-term magma supply rate that injects magma into a shallow magma chamber and consequently controls the erupted volume, even though this magma is not continuously erupted at the surface.) Such an inferred period of activity is probably shorter than the one that actually occurred, because crater counts for the flanks of the volcano²⁰ show that Alba Patera had a very long period of activity, extending over perhaps 1.0–2.0 billion years. If these time periods are correct, then the observed volume of volcanic material at Alba Patera would, on average, have been erupted at a much slower rate, equivalent to only 10–20% of the above high magma supply rate (10⁻³–2 × 10⁻³ km³ yr⁻¹) and 25–50% of the low rate (2.5–5.0 × 10⁻⁴ km³ yr⁻¹). These average supply rates are approximately equal to the magma production rate of the Central Andes

Table 1 Water-driven ignimbrite eruptions on Alba Patera

Magma water content (wt %)	Minimum magma mass flux (kg s ⁻¹)	Minimum water mass flux (kg s ⁻¹)	Time to erupt 57,700 km ³ magma (yr)	Time to erupt 60 km ³ of magma (days)	Min. total water to atmos. (mm)	Min. atmos. water per event (μm)
0.5	3.0×10^7	1.5×10^5	166.6	63.0	5.1	5.3
1.0	1.5×10^8	1.5×10^6	35.8	12.6	10.2	10.7
2.0	7.0×10^8	1.4×10^7	7.1	2.7	20.7	21.3
4.0	5.0×10^9	2.0×10^8	1.0	0.4	41.3	42.6
5.0	1.0×10^{10}	5.0×10^8	0.5	0.2	51.7	53.3

over the past 20 million years¹⁹ and do, of course, have important implications (not addressed here) for (1) the geometry of the dike systems that allows magma to ascend from the mantle to the near-surface; and (2) the ability of the summit magma chamber to survive thermally for an extended period of time (millions of years).

The calculated volume of ash, as distinct from lava flows, on the flanks of Alba Patera is constrained by the minimum thickness of the ash layer(s) within which the channel networks have been carved (estimated from shadow measurements to be 150 m deep and extending out to a radial distance of 450 km from the summit; ref. 5). This volume is therefore $\pi \times 450^2 \times 0.15 \approx 10^5$ km³ which, choosing a plausible density of, say, 1,500 kg m⁻³, corresponds to a dense rock (2,600 kg m⁻³) volume equivalent to 57,700 km³. This dense rock volume is nearly 1,000 times the volume of one of the sub-calderas, given the uncertainties in determining these values. If the total volume of pyroclastic flows and associated co-ignimbrite fall units were spread out uniformly, and the same amount of material were erupted each time, then a layer ~150 m deep (the minimum thickness based on the channel depths) would represent 1,000 eruptions, each producing a 15-cm-deep layer. As an alternative, if the ash were spread over one-third of the flanks each time, say, then each layer would be ~0.5 m deep.

Because pyroclastic flows (ignimbrites) were formed in the explosive eruptions of Alba Patera, rather than high-convecting eruption clouds leading to ash-fall deposits, this enables us to set a lower limit on typical eruption rates. To form pyroclastic flows, that is, for a convecting cloud to be unstable, the mass flux must be greater than some limiting value for any chosen magma volatile type and content^{21,22}. We have modelled such situations numerically, for the cases where the volatile is water (Table 1) or carbon dioxide (Table 2), calculating the minimum mass flux required to ensure ignimbrite formation, the corresponding maximum time needed to erupt the observed deposits, and the corresponding volumes of associated volatiles erupted.

If we adopt a value for the magma water content of between 0.5–5.0 wt%, then it is evident that continuous activity lasting from at most ~167 yr (at 0.5 wt% H₂O) to six months (at 5.0 wt%) would be required to produce the entire dense rock equivalent of the Alba Patera pyroclastic materials. For individual caldera-forming events, these figures are ~63 days

and 5 h respectively if our estimate of 60 km³ for the volume of each caldera-forming event is valid. Greeley¹ has calculated that all of the martian volcanic constructs would have outgassed the equivalent of 1.47 m of water over the surface area of the planet during the whole of martian geological history following the period of heavy bombardment (plains volcanism would have added an additional ~8.6 m during the same time interval). Table 1 indicates that Alba Patera would have contributed between ~5 mm–5 cm depth of water to this estimate of the released volatiles during its entire lifetime if the magma water content was 0.5–5.0 wt%, respectively. Equivalent contributions from individual caldera-forming events would have been ~5 μm–50 μm, with corresponding minimum eruption rates of ~0.1–300 μm per day, respectively.

We stress here that the eruption rates in Tables 1 and 2 are minimum values; by analogy with the conduit geometry known to occur during large terrestrial explosive eruptions²³ we expect actual discharge rates to be one to two orders of magnitude greater than these minimum values. This would lead to planet-wide equivalent water depth accumulation rates of 10²–10³ μm per hour for time intervals ranging from tens of minutes to hours. The precise areal distribution of ice crystals condensing from the erupted water is uncertain, due to our inadequate knowledge of paleometeorology on Mars, but we note that some models²⁴ may indeed produce a deposit of global extent. The extreme alternative situation would be that the water vapour would condense in the immediate vicinity of the volcano, the resulting ice crystals being incorporated in the near-vent fall deposits⁵. Such a volatile distribution would not be sufficient to explain the formation of the observed channel networks on the flanks of Alba Patera⁵.

The same type of calculation can be performed by assuming that carbon dioxide was the main volatile species driving the explosive activity on Alba Patera. Table 2 provides the corresponding minimum magma and CO₂ mass fluxes, and the time to erupt both the total mass of pyroclastics and the volume equivalent of a single caldera, together with the resultant minimum mass fractions of the total present-day atmospheric mass of Mars (~2.7 × 10¹⁶ kg; S. E. Postawko, personal communication) and the mass fraction per eruptive event. Note from Table 2 that the total maximum duration of the activity is longer (by a factor of ~3) than was the case for water, while the

Table 2 Carbon dioxide-driven ignimbrite eruptions on Alba Patera

Magma CO ₂ content (wt %)	Minimum magma mass flux (kg s ⁻¹)	Corresponding Min. CO ₂ mass flux (kg s ⁻¹)	Time to erupt 57,700 km ³ magma (yr)	Time to erupt 60 km ³ magma (days)	Min. % of total atmos. mass	Min. atmos. mass frac. per event (p.p.m.)
0.5	1.0×10^7	5.0×10^4	475.8	179.0	2.6	29
1.0	3.5×10^7	3.5×10^5	136.0	51.2	5.2	58
2.0	1.2×10^8	2.4×10^6	40.0	14.9	10.6	116
4.0	5.0×10^8	2.0×10^7	9.5	3.6	21.2	232
5.0	9.0×10^8	4.5×10^7	5.3	2.0	26.4	289

Table 3 Constraints on water-supply-driving eruptions

Magma water content (wt %)	Minimum magma mass flux (kg s ⁻¹)	Corresponding minimum water mass flux (kg s ⁻¹)	Corresponding minimum water volume flux (m ³ s ⁻¹)	Horizontal dike length (km)	Vertical aquifer thickness (km)
0.5	3.0×10^7	1.5×10^5	1.5×10^2	2.7	0.9
1.0	1.5×10^8	1.5×10^6	1.5×10^3	4.1	0.6
2.0	7.0×10^8	1.4×10^7	1.4×10^4	6.0	3.9
4.0	5.0×10^9	2.0×10^8	2.0×10^5	9.8	34.1
5.0	1.0×10^{10}	5.0×10^8	5.0×10^5	11.6	71.7

minimum mass of erupted CO₂ is very large compared to the current mass of the martian atmosphere: 2.6% for a CO₂ content of 0.5 wt% in the magma, and 26% of the atmosphere for a CO₂ content of 5.0 wt% in the magma. An important constraint on the possible CO₂ content of the martian magma is the solubility of CO₂. Almost exactly 1 wt% can dissolve per 50 km of depth of the source region in the martian interior¹³. Based on models of the evolution of the thickness of the martian elastic lithosphere²⁵, we do not expect magma source depths to be > 50 km in the Alba Patera region at the time of these eruptions, thereby constraining the CO₂ content of the parental magma to be ≤ 1 wt% CO₂ and implying that the eruptions of Alba Patera released ~5% of the current martian atmosphere mass.

We have also investigated the possibility that the Alba Patera eruptions may have recycled meteoric water to drive the explosive activity rather than parental water. We can work out the vertical extent of a possible aquifer feeding the magma plumbing system provided we make some simple assumptions about the geometry^{13,26}. The presence of an elongate dike at depth feeding the surface vent will allow easiest water access to the magma. The required parameters are the horizontal length L and width W of the dike (strictly speaking below the depth where magmatic volatiles exsolve—a few hundred metres for water; if the aquifer is shallower than this it will make little difference to our calculations because the widening of the fissure towards the surface that is expected to take place to accommodate the exsolving gas will be accomplished more by an increase in W than in L) and the vertical extent H of the aquifer. The water volume flux V into (both sides of) the dike is then:

$$V = 2HLPV$$

where U is the water velocity and P is the porosity of the aquifer. We can assume generous values of $P = 30$ volume % and $U = 10^{-3}$ m s⁻¹ (ref. 23), and obtain values of V from the water mass fluxes in Table 1 (by dividing by the water density). It remains to specify values of L for each eruption rate; these come from the shape and size of dikes needed to accommodate the appropriate magma mass flux. Again, such a situation has been investigated for both martian¹³ and terrestrial²⁷ volcanoes. Using general conduit geometry/magma supply rate relationships²⁸, the required length, L , is given by:

$$L = (12\mu MZ^3/\beta g\Delta)^{0.25}$$

where μ is the magma viscosity, β the magma density, g the acceleration due to gravity, Δ the density difference between magma and country rock, M the mass flux and Z is the ratio L/W . Z is introduced because it is known that, whatever the individual values of L and W may be, L/W will probably be in the range 100–1,000 (ref. 29). We can therefore use values (appropriate to mafic magmas) of $\mu = 300$ Pa s, $\beta = 2,600$ kg m⁻³, $g = 3.8$ m s⁻², $\Delta = 200$ kg m⁻³, and take $Z = 1,000$ as this gives us the smallest implied water flux in the aquifer. Table 3 repeats the mass fluxes from Table 1 for reference, gives the aquifer water volume flux derived from the water mass flux, then gives the dike length L required by the magma mass flux,

and finally gives the vertical aquifer thickness required to supply the dike with the correct water flux.

Given that, without special, localized, volcanic-heating events taking place, we do not expect liquid water to be typically available in large volumes at depths ≥ 2–4 km due to lithostatic pressure and the depth of the permafrost layer^{30,31}, this implies that only eruptions at mass fluxes of ≤ 4×10^8 kg s⁻¹ can have any appreciable contribution to their volatile phase from meteoric water. All those values at higher mass fluxes (where water is the dominant volatile) must be driven almost entirely by juvenile water. Because we expect, as stressed earlier, that ignimbrite eruptions do commonly occur at these and even higher eruption rates on Mars^{12,13}, it follows that we expect such eruptive events to be major contributors of juvenile water to the atmosphere/regolith water budget. What remains to be solved (perhaps by detailed crater counts of high-resolution images unavailable at the time that Neukum and Hiller²⁰ performed their analysis) is the duration of time over which these explosive eruptions occurred. It is clear, however, that the volatile input from volcanoes such as Alba Patera could have had a major role in the modification of the global volatile inventory^{3,4,32}, and that this input continued into the relatively recent (~past 2 Gyr) geological history of Mars.

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Thermally activated release of stored chemical energy in cryogenic media

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We have observed very rapid spontaneous heating of the cold solid methane moderator when irradiated by fast neutrons at low temperatures in the Intense Pulsed Neutron Source. In one instance enough heat was released to vaporize a fraction of the methane. We have also induced smaller rapid heat releases—both by slowly warming irradiated methane and by diminishing the effectiveness of cooling. An explanation for these observations is that energy stored in the form of reactive species (H , CH_3 , ...) accumulates in solid methane irradiated at low temperatures, and is released by thermally activated diffusion and subsequent reaction. During irradiation, the density of reactive species can build up sufficiently to become unstable as the rate of heat release due to recombination exceeds the rate of heat loss to the cooled surroundings. Similar effects may occur in other cryogenic moderator materials, and may be responsible for the jets observed from cometary nuclei.

In the Intense Pulsed Neutron Source (IPNS), a beam of 450 MeV protons, pulsed at 30 Hz, produces neutrons in a spectrum peaked at ~ 1 MeV by spallation and fission from a depleted uranium target. Unless stated otherwise, all rates in this letter refer to an average proton current of 15 μA corresponding to a neutron flux, between 1 eV and 1 MeV in energy of $\sim 5 \times 10^{12}$ neutrons $cm^{-2} s^{-1}$ in the moderators. The nuclear heating power is about 20 W, corresponding to a dose rate of 2×10^3 rad s^{-1} or 1.2×10^{17} eV $g^{-1} s^{-1}$, mostly due to fast neutrons. The cold solid methane moderator of the IPNS is an aluminium box filled with solid methane and 6% dense aluminium metal foam for heat transfer purposes, cooled by contact with a cold helium loop welded to the container¹. It slows down fast neutrons from the primary source to provide beams of low energy neutrons for scattering and reflection measurements. The assembly can be maintained at temperatures as low as 7 K even while irradiated by the nearby fast neutron source. A thermocouple near the centre registers the temperature. The system performs effectively, but its operation has been marred by roughly periodic spontaneous temperature excursions during or shortly after continuous irradiation, some of which have damaged the methane container². These are now under control.

Figure 1 shows a rapid rise in temperature purposely induced by throttling down the coolant flow rate after 3 days of irradiation at 14 K with $\sim 5 \times 10^{12}$ neutrons $cm^{-2} s^{-1}$. The temperature rose to 40 K in about 20 seconds. The heat released (calculated from the temperature rise, the known masses of Al (690 g) and CH_4 ,

(314 g; 19.6 moles) and their heat capacities and enthalpies), was ~ 15 kJ. In one unrecorded spontaneous event that destroyed a moderator container, the temperature rose sharply from 9 K to 110 K (where methane vaporizes at 1 atmosphere pressure). This occurred soon after shutting down the primary source after 21 days of irradiation at 9 K. The heat released must have been > 100 kJ. On average, the results of several measurements like the one shown in Fig. 1 indicate that stored energy builds up at a rate of about 0.5 kJ h^{-1} . After temperature excursions, H_2 is seen in the cover gas above the moderator.

Either the aluminium or the methane could be responsible for the observed effects. Radiation damage energy accumulates in aluminium as Frenkel (vacancy-interstitial) pairs. For example, the metal accumulates 22 J g^{-1} after a fluence of 10^{19} neutrons cm^{-2} at 4.6 K, which corresponds to saturation (R. C. Birtcher, personal communication). We observed about 1.1 J g^{-1} released in the empty aluminium box irradiated for one day at 21 K, roughly consistent with this figure. The damage energy in aluminium is not enough to explain the result of Fig. 1 or the catastrophic release which destroyed a moderator container. Thus the methane must be involved.

Fast neutron collisions, and the ensuing damage cascade in irradiated methane produce monatomic hydrogen, CH_3 radicals and other reactive species, some of which accumulate unreacted in the interstices of the solid CH_4 . After one week's irradiation, we vaporized the contents of the moderator. (After warming, any reactive species have reacted.) Gas chromatography analysis indicated about 5.4 mol% H_2 and 2.3 mol% C_2H_6 , with smaller concentrations of other hydrocarbons. Table 1 shows the distribution of radiolysis products¹. A crude calculation based on the fraction of evolved hydrogen and the estimated n-p collision rate indicates that about 500 protons are produced in the damage cascade per n-p collision. As the reaction $H + H \rightarrow H_2$ releases 218 kJ mol^{-1} H_2 , accumulation of 5.4 mole % per week represents an energy storage rate of 1.4 kJ h^{-1} . Similarly, $CH_3 + CH_3 \rightarrow C_2H_6 + 368$ kJ mol^{-1} C_2H_6 represents 1.0 kJ h^{-1} . Ignoring $H + CH_3 \rightarrow CH_4$ and other reactions, these two represent an energy storage rate substantially greater than the observed rate, 0.5 kJ h^{-1} . These figures are based on the assumption that all the energy represented by H_2 and C_2H_6 evolved after warmup is stored as H or CH_3 in the quiet periods between releases. These are certainly overestimates, in view of both the inhomogeneity of the damage cascade and the consequent rapid initial annihilation of defects, and of the gradual annihilation at low temperatures. Reasoning from this close relationship between the energy equivalent of the radiolysis products and the calorimetrically observed energy release, we assert that the released energy is stored as chemical energy in H and CH_3 , although energy stored in lattice defects or distortion remains a possibility whose magnitude we cannot assess.

Assuming $(368 + 218)/2 = 293$ kJ mol^{-1} CH_4 is stored as H and CH_3 , the energy storage rate of 0.5 kJ h^{-1} corresponds to a rate

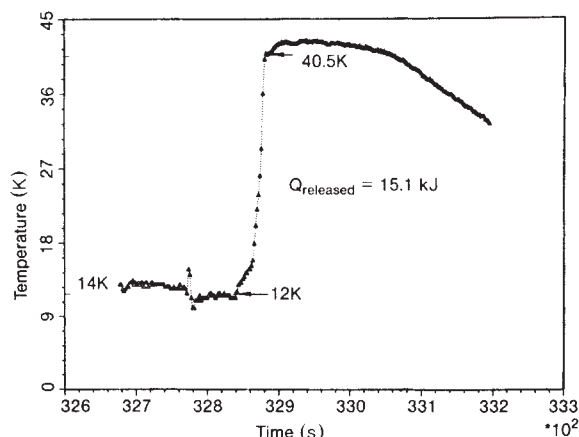


Fig. 1 The record of a temperature excursion of the IPNS solid CH_4 moderator, after three days' irradiation with fast neutrons at 5×10^{12} neutron $cm^{-2} s^{-1}$, induced by throttling the He coolant flow. The temperature at first decreased when the irradiation was stopped and as the coolant expanded; the system became unstable because the heat transfer became less effective at the lower flow rate. The long recovery time is due to leakage of H_2 into the insulating space and refrigeration system transients.

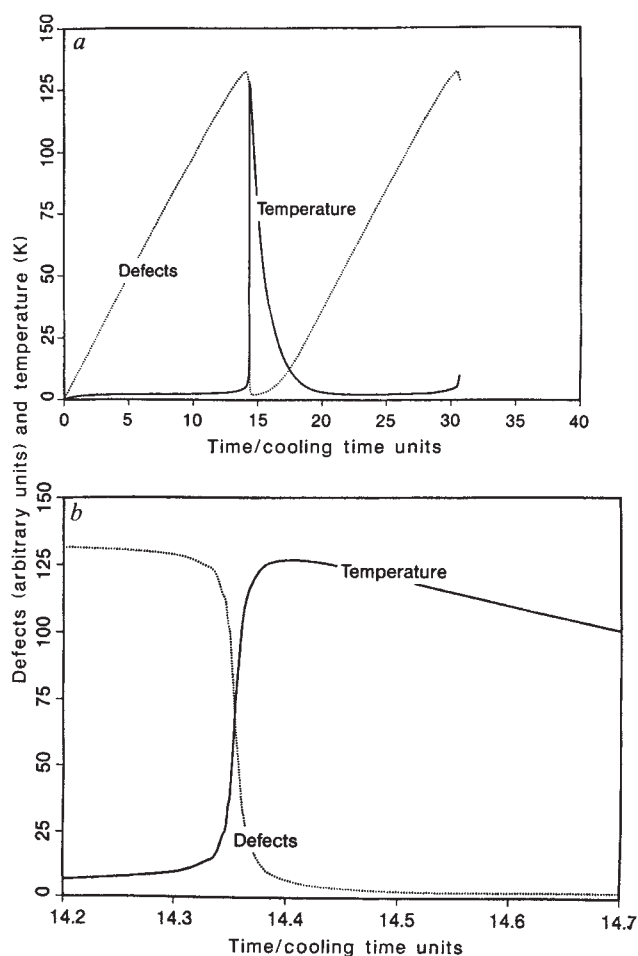


Fig. 2 *a*, Runge-Kutta integration of the annealing equations, with arbitrarily chosen constant coefficients MC , U , ΔE , ϵ , R , P , K_∞ and T_{cool} (see text), chosen to produce instability. The system acts as a nonlinear oscillator. Panel *b* shows the excursion in detail.

of build-up of H and CH_3 of $0.5/293 = 1.7 \times 10^{-3}$ mol H or CH_3 per hour, or $1.7 \times 10^{-3}/19.6 = 8.7 \times 10^{-5}$ mol (H or CH_3) h^{-1} mol $^{-1}$ CH_4 .

Atomic H and CH_3 diffuse in the methane crystal lattice. If the rate of diffusion and consequent reaction depends strongly enough on the temperature, the concentration of H and CH_3 can build up at low temperatures leading to thermally-triggered release and instability. Describing the reaction rate as a binary collision process involving only one species, with a rate coefficient having Arrhenius-law temperature dependence, with heat removal to the coolant represented in newtonian form, and with a lumped-parameter description of the system, simple equations describe the build-up and disappearance of defects (H, CH_3 and so on):

$$dN(t)/dt = R(t) - K(T)N^2(t) \quad (1)$$

$$MC(T)dT/dt = P(t) + \epsilon K(T)N^2(t) - AH(T, t)(T(t) - T_{\text{cool}}(t)) \quad (2)$$

$$K(T) = K_0 \exp(-\Delta E/k_B T) \quad (3)$$

Here, N is the number of defects, R is their production rate, and K is the (volume) recombination rate coefficient. M is the mass of the system, $C(T)$ its specific heat at temperature T , P the sensible heating power due to the external source, ϵ the heat released per defect annihilation, H the heat transfer coefficient and A the contact area between the system and the

Table 1 Analysis of volatile products from radiolysis of methane from the solid methane moderator

Product	Mole fraction (%)
H_2	5.38
CH_4	91.37
C_2H_4	0.20
C_2H_6	2.30
C_3H_8	0.42

coolant. T_{cool} is the temperature of the coolant, ΔE is the activation energy for defect diffusion and K_0 the recombination rate at infinite temperature. The equations are not novel, and are intractably nonlinear. They are the equations of annealing kinetics; for example they have been invoked to describe the Wigner energy release in irradiated graphite³, which is similar in its kinetics to the present phenomenon, although different in the nature of the energy storage mechanism⁴. They are a simplified form of the theory of thermal explosions^{5,6}, but in this case include an external source term for defects as well as for heat. A complicating feature here is that because of the low temperatures the coefficients C and H are strong functions of the temperature.

If the defect production rate and the temperature are constant, equation (1) is decoupled from equation (2) and the number of defects increases in a simple way: $N(t) = N_\infty \tanh(t/\tau_N)$, where $N_\infty(T) = \sqrt{R/K(T)}$ and $\tau_N(T) = 1/\sqrt{RK(T)}$. When the defect production rate is zero and the temperature is constant, the number of defects decreases from an initial value $N(0)$ as $N(t) = N(0)/(1 + N(0)K(T)t)$. When the number of defects is so large that an incremental increase of the temperature causes an increase in the rate of release of stored damage energy which is greater than the increase in the rate of heat removal to the surroundings, the temperature rises uncontrollably. Ever-increasing temperatures lead to ever-more rapid diffusion and annihilation until the stored energy is depleted when

$$\epsilon N^2(dK(T)/dT) = \epsilon N^2 K(T)(\Delta E/k_B T^2) > d/dT(U(T)(T - T_{\text{cool}})) \approx U(T) \quad (4)$$

that is $N^2 \geq U(T)k_B T^2/(\epsilon K(T)\Delta E)$, where $U = AH$. Assuming the temperature and defect production rate to be constant up to the time of the instability, (more generally, the temperature during irradiation and the instantaneous temperature must be distinguished), the time t_s required until N exceeds the value above is given by $N_\infty^2(T) \tanh^2(t_s/\tau_N(T)) = U(T)k_B T^2/(\epsilon K(T)\Delta E)$, or

$$t_s = \tau_N(T) \tanh^{-1}[\sqrt{U(T)k_B T^2/(N_\infty^2(T)\epsilon K(T)\Delta E)}] \quad (5)$$

We have determined an approximate value for the activation energy. If the argument of the hyperbolic tangent is small, and if U is independent of T , the ratio of times to reach unstable conditions at two temperatures is $t_{s1}/t_{s2} \approx T_1/T_2 \exp[(\Delta E/2k_B)(1/T_1 - 1/T_2)]$. Spontaneous temperature excursions occurred regularly at intervals of about 24 h at $T = 14$ K, and the destructive one took place after 336 h at 9 K under otherwise nearly identical irradiation conditions. Thus we estimate

$$\Delta E/k_B = 2 \ln[(t_{s1}/t_{s2})(T_2/T_1)](1/T_1 - 1/T_2)^{-1} \approx 155 \text{ K} \quad (6)$$

This large an activation temperature is required to explain the unstable behavior of the system, for then it follows that when the temperature is low, small changes in the temperature make large changes in the defect annihilation rate. According to Eyring's theory of self-diffusion, that the activation energy is equal to the sublimation energy per molecule (about $10,000 \text{ J mol}^{-1}$ in methane) $\Delta E/k_B$ would be about 1,200 K.

That the present result is substantially lower may implicate H as the triggering species.

When $R \neq 0$, the equations exhibit either stable or nonlinear oscillatory behavior according to whether the argument of equation (5) is greater than or less than one. Stability is guaranteed if $U(T)k_B T^2/[N_\infty \varepsilon \Delta E K(T)] = U(T)k_B T^2(\varepsilon R \Delta E) > 1$.

We have integrated equations (1)–(3) with a constant source of defects and coolant temperature and temperature-independent thermal properties, by a Runge-Kutta method. For one set of (guessed) parameters the methane temperature and defect number rose smoothly to level values. For another set corresponding to lower coolant temperature, the temperature shot up rapidly after a slow smooth rise and the defect number plummeted; the temperature decreased again and the behavior repeated itself in a nonlinear oscillatory fashion similar to the physical observation. Figure 2 shows the oscillatory solution.

This mechanism of energy storage and release may also operate in other situations where chemically active species accumulate at low temperatures and recombine to release heat in a process of thermally activated diffusion. Hydrogen and hydroxyl radicals in irradiated ice and heavy ice, and H and hydrocarbon radicals in polyethylene moderators at cryogenic temperatures may behave in this way. Also, reactive radicals, molecular fragments, and atomic hydrogen condensing at low temperatures on cometary nuclei distant from the Sun, or produced by energetic particle irradiation, could store energy in this way. Upon warming, and by the mechanism described here, the energy released may vaporize material, giving rise to jets such as have been observed from Halley's and other comets, and might accelerate large solid particles like those observed in cometary environments⁷. In such cases, the theory of combustion, including the effects of spatial diffusion of heat and defects, would have to be adduced.

Recently, Brown and his colleagues have reported studies of the emission of various molecules from ion-irradiated, cold D₂O ice and cold solid CD₄ (refs 8,9). The release of D₂ from CD₄ exhibits a sharp onset similar in appearance to the temperature excursions we observed in the IPNS CH₄ moderator, but appears to require a different explanation. Cold solid methane moderators in the Japanese pulsed spallation neutron source, KENS, originally did not suffer temperature instabilities, until the source intensity was increased¹⁰. Studies of the effects of gamma-ray irradiation some years ago uncovered an energy storage mechanism like the present one, but the systems became unstable at 4 K at concentrations of only about 0.2 mol% H or CH₃, much lower than what we infer from our calorimetry. Presumably all these observations and their variance from the present ones could be explained by differences in defect production rate, the nature of the radiation (neutrons or gamma-rays), cooling effectiveness or temperature.

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Synthesis and structure of (*cis*)-[1-ferrocenyl-2-(4-nitrophenyl)ethylene], an organotransition metal compound with a large second-order optical nonlinearity

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There is considerable interest in materials with nonlinear optical properties. Two important manifestations of light interacting with a nonlinear optical material are, the process of second-harmonic generation (SHG) which is relevant to new laser technology, and the electro-optic effect which has applications in telecommunications and in integrated optics¹. Several inorganic and organic materials with unusually large second-order nonlinearities are known¹. However, reports of organometallic compounds with second-order nonlinear optical properties are few^{2–4} and they have relatively small values for SHG efficiencies. Here we describe a new compound (*cis*)-[1-ferrocenyl-2-(4-nitrophenyl)ethylene] (compound 2) exhibits solvatochromic behaviour. A Kurtz-powder measurement, using 1.907 μm light from a Raman shifted Nd-YAG laser operating at 1.064 μm , shows the compound provides highly efficient second harmonic generation, with a signal some 62 times that from a urea reference sample. The single crystal X-ray structure of (*cis*)-[1-ferrocenyl-2-(4-nitrophenyl)ethylene] has been determined. The large nonlinear optical property of this compound demonstrates that organotransition metal compounds can play an important role in the development of the nonlinear optical properties of materials.

Table 1 illustrates organic compounds with large nonlinear optical properties. Such materials have a large molecular second-order hyperpolarizability, which is related to charge-transfer

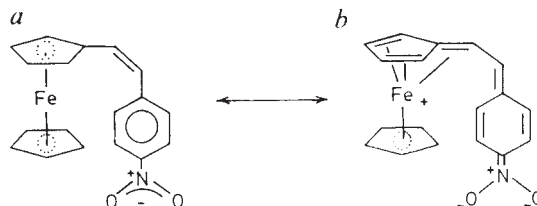


Fig. 1 Two canonical representations of 2. *Data characterizing compound 2:* ¹H NMR data, in [²H]CHCl₃ at 300 MHz [given as chemical shift (in p.p.m. relative to solvent as the internal standard) (relative intensity, multiplicity, coupling constant (in Hz), assignment). The data are 8.13 (2H, d, *J* = 8.8 Hz, CHCHCHO₂), 7.50 (2H, d, *J* = 8.8 Hz, CHCHCNO₂), 6.53 (1H, d, *J* = 11.9 Hz, *n*-C₅H₄-CH), 6.41 (1H, d, *J* = 11.9 Hz, *n*-C₅H₄-CH = CH), 4.25 and 4.17 (two bands of relative intensity two, each band is of three lines, an 'apparent triplet' with separation between the components of 1.8 Hz, assigned as an AA'BB' complex band arising from the 4H of the η -C₅H₄ ligand), 4.13 (5H, s, η -C₅H₅). Mass spectrum: *M/e* = 333, corresponding to the parent ion [2]⁺. Microanalytical data: Found: C, 64.59; H, 4.55; N, 4.22%; calculated for C₁₈H₁₅FeNO₂: C, 64.89; H, 4.54; N, 4.21%].

Table 1 Second-harmonic generator (SHG) powder efficiencies of selected organic compounds by urea

Compound	SHG	Compound	SHG
	1		70
	7.5		80
	8.8		80
	10.0		115
	13		160
	22.0		250*
	25.0		

Efficiency was measured using the Kurtz technique at 1.064 μm relative to urea.

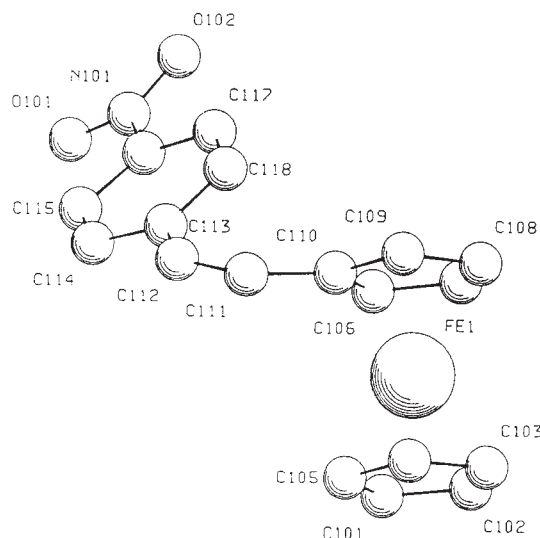
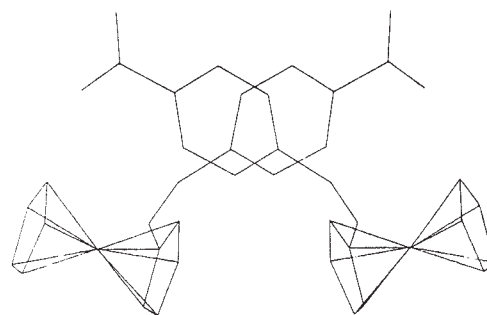
*Measured at 1.907 μm .

ability and electron delocalization via π -electron conjugation in the molecule¹. It has been noted that there is a correlation between the solvatochromism of a molecule and the magnitude of the molecular second-order polarizability (β)⁵.

Crystals of molecules with large values of β will exhibit no second-order nonlinearities if the crystal space groups are centrosymmetric because symmetry dictates that in these cases the bulk susceptibility χ^2 will be zero. Therefore a non-centrosymmetric crystal structure and a large molecular hyperpolarizability are critical considerations for the design of materials with large second-order nonlinearities.

One possible advantage of introducing organotransition metal groups lies in their potential for redox behaviour, a property largely associated with the metal centre. Facile redox ability can be envisaged to lead to large hypopolarisability. On this basis, and drawing on chemical analogy with the organic compounds shown in Table 1, we initiated an exploratory synthesis programme to prepare organotransition metal compounds exhibiting large second-order nonlinearities. We focused on ferrocene derivatives which have an extensive organic chemistry and normally show low sensitivity to di-oxygen and water.

Deprotonation of $\{(\eta\text{-C}_5\text{H}_5)\text{Fe}(\eta\text{-C}_5\text{H}_4\text{CH}_2\text{P}(\text{C}_6\text{H}_5)_3)\}\text{I}$ (compound 1; see ref 6) with *n*-butyl lithium, followed by addition of 4-nitrobenzaldehyde, yielded dark purple crystals of (*cis*)-[1-ferrocenyl-2-(4-nitrophenyl)ethylene] (compound 2)⁷ and the previously reported (*trans*)-[1-ferrocenyl-2-(4-nitrophenyl)ethylene] (compound 3)⁷. The compounds 2 and 3

**Fig. 2** One of the molecules in the asymmetric unit for **2**. Hydrogen atoms have been omitted for clarity.**Fig. 3** View of the two molecules of **2** comprising the asymmetric unit.

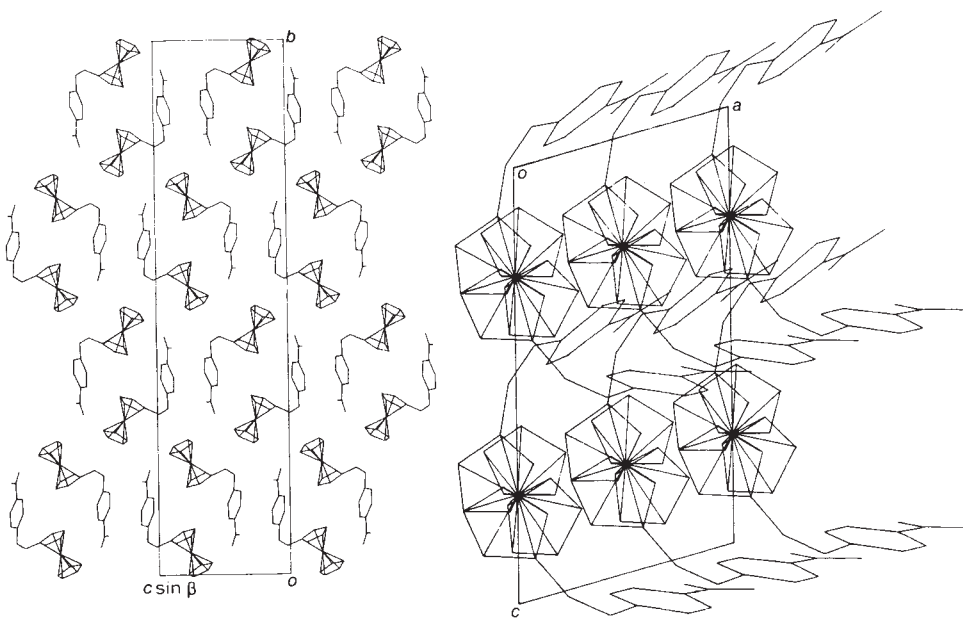
were readily separated by chromatography on alumina. Starting from **1** (2.94 g) the yield of **2** was (0.50 g), 30%. Solutions and crystals of **2** appear to be stable indefinitely in air.

The solution structure proposed for **2** is shown in Fig. 1a. It was anticipated that **2** would have a substantial hyperpolarizability by virtue of the known redox ability of ferrocene derivatives, as indicated by the varying charge distributions of the canonical representations of **2** shown in Fig. 1. Compound **2** should therefore have a highly charge correlated excited state which should lead to the large solvation effects, as observed by optical spectroscopy.

In accord with this expectation, the solution ultraviolet visible spectrum of **2** was markedly affected by varying the solvent. In heptane there were three absorptions at 320, 406 and 462 nm whereas in *N,N'*-dimethylformamide, there were only two absorptions at 340 and 492. In view of the solvatochromic behaviour of **2**, it was tested for SHG by the Kurtz powder technique⁸. Because of the intense optical absorption of the crystals of **2**, light from a Nd-YAG laser, Raman shifted to 1.907 μm using a high pressure hydrogen cell, was used. This resulted in frequency doubled light emitted at 0.953 μm which had an intensity 62 times that of the urea reference sample. In contrast, **3**, the *trans* isomer of **2**, showed no SHG signal and this suggests that **3** crystallizes in a centrosymmetric space group.

A single crystal X-ray diffraction study of **2** was performed on a crystal obtained from slow evaporation of a saturated solution of 2-propanol. Crystals of **2** obtained in this way crys-

Fig. 4 Packing of **2** viewed along the *a* axis (left) and *b* axis, showing clearly the polar nature of the crystal structure. *Crystal data:* $C_{18}H_{15}FeNO_2$, $M = 333.2$, monoclinic, space group Cc, $a = 6.015(3)$, $b = 44.547(10)$, $c = 11.552(6)$ Å, $\beta = 105.11(6)^\circ$, $U = 2988.4$ Å³, $z = 8$, $D_c = 1.48$ mgm⁻³, $\lambda(\text{Mo-K}\alpha_1) = 0.70930$ Å, $\mu(\text{Mo-K}\alpha_1) = 10.12$ cm⁻¹, $F(\text{OOO}) = 1376$. Crystal dimensions $0.425 \times 0.1 \times 0.075$ mm. At convergence $R = 0.065$ $R_w = 0.070$ for 1,734 reflections [$I > 3\sigma(I)$] in the range $1 < \theta < 25^\circ$. Iron, nitrogen and oxygen were refined anisotropically, carbon isotropically and hydrogen in calculated positions riding on their attached carbons. The cyclopentadienyl rings were refined subject to soft vibrational restraints (refs 10 and 11). All calculations were performed on the VAX 11/750 computer of the chemical Crystallography Laboratory, Oxford using the Oxford CRYSTALS package¹². $R = \sum (|F_o| - |F_c|) / \sum |F_o|$, $R_w = \sum (|F_o| - |F_c|)^2 / \sum |F_o|^2$.



tallize in the non-centrosymmetric space group Cc. Unfortunately the crystal quality was not sufficiently good to allow a detailed study of molecular geometry but was certainly good enough to give a reasonable picture of atomic positions and crystal packing.

The molecular structure of **2** is shown in Fig. 2 with labelling. The asymmetric unit (shown in Fig. 3) contains two independent molecules of **2** related by a noncrystallographic twofold axis. We note that the nitrophenyl ring and η -C₅H₄ ring of each independent molecule are not coplanar, thereby minimizing unfavourable steric interactions between protons of these groups (interplanar angles are 132.5 and 132.8°). The nitro groups are slightly twisted out of the phenyl ring planes by 8.1° and 8.4° and in addition the C(109)-C(110)-C(111)-C(112) torsion angles are 151.2° and 152.2° and C(111)-C(112)-C(113)-C(114) torsion angles are 155.0° and 154.4° for the two molecules respectively. Figure 4 shows the packing of molecules in the crystal lattice viewed along the *a* and *b* axes, respectively. From Fig. 3 and 4 it is clear that the molecular dipole of **2** will be preserved to a substantial extent in the crystalline material. Indeed the tilting of the molecular dipoles will favour efficient phase-matched second harmonic generation⁹. Precise comparison with the theoretical predictions is not possible because the exact orientation of the molecular dipole and the related linear and second-order polarizabilities are not known.

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North Pacific deep water formation during the latest glaciation

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For at least 20 years palaeoceanographers have speculated about the existence of a source of young (nutrient-depleted) deep water in the North Pacific (NPDW) during glaciations. Proof of its existence has eluded researchers because the present deep North Pacific is very corrosive to calcium carbonate. Thus, it has been difficult to obtain a long time series of oxygen isotope data on benthic foraminifera for dating the sediment, and carbon isotope data for use as a proxy for deep water nutrient content. I report here on a stable isotope record from a western subarctic Pacific core taken at 3 km water depth. The carbon isotope ratios in the benthic foraminifera *Cibicidoides* show no evidence of nutrient-depleted NPDW from glacial to Holocene time.

In the modern North Pacific there is no deep water formation because surface waters are too fresh, which produces stable stratification. The low surface salinity of the North Pacific is thought to be due to the low rate of evaporation, which may be related in part to low inflow of warm surface waters from the south¹. Rather than deep water, an intermediate watermass is formed in the North Pacific, and it is traced to middle and low latitudes at depths down to 1 km (ref. 2). The mechanism of this watermass formation is probably vertical diffusion; even in winter-time, convection in the north-west Pacific is limited to about 150 m (ref. 3).

Many studies have documented dramatic changes in deep ocean circulation during glacial time. Palaeochemical evidence points toward decreased production of North Atlantic Deep Water relative to Antarctic bottom water in the Atlantic Ocean⁴⁻⁸. Broecker *et al.*⁹ speculated that there may actually be two modes of ocean circulation, with the Atlantic and Pacific Oceans becoming more similar in glacial time. Some support for this

hypothesis has recently come from the discovery of a nutrient-depleted watermass at intermediate depths in the glacial North Atlantic and below 1,600 m in the Caribbean¹⁰⁻¹³. So far, however, the most convincing evidence for glacial ocean circulation change has come from the Atlantic.

Nutrient-depleted NPDW was first proposed to account for generally better preservation of calcareous microfossils of glacial age compared to interglacial (core top) microfossils in the equatorial Pacific¹⁴. Subsequently it has been hypothesized that regional differences in patterns of Pacific carbonate accumulation might have resulted from glacial NPDW formation in the western subarctic Pacific¹⁵. Most recently, four groups of authors have prepared manuscripts which bear on the NPDW problem. One group describes oxidized layers in cores of dominantly reduced lithology from the deep North Pacific, concluding that this facies change is due to glacial-interglacial changes in oxygenation of bottom waters, resulting from NPDW production (W. E. Dean and J. V. Garder, personal communication). Carbon isotope data from Pacific Ocean sediment cores suggest that during glacial times an oxygen-rich watermass did indeed exist, but it was restricted to the depth range 700 to 2,600 m (J.-C. Duplessy *et al.*, in preparation). This result is in general agreement with the modelling of radiocarbon data which suggests that NPDW extended somewhat deeper than 2 km during glacial times (W. H. Berger, personal communication). A synthesis of pooled oxygen and carbon isotope data on glacial and Holocene *Cibicidoides*, shows greater variability in glacial than interglacial $\delta^{13}\text{C}$ in the Pacific Ocean, suggesting that the glacial hydrography of the Pacific was more complex than today (W. B. Curry, personal communication). Curry *et al.* also report that lowest $\delta^{13}\text{C}$ values 18,000 years ago were found in southern ocean cores, which suggests that some nutrient-depletion occurred in the Pacific Ocean at their core locations.

Of all the above studies none has $\delta^{13}\text{C}$ data from the deep northern-most Pacific. Meiji Tongue, an elongate, thick pile of hemiterigenous sediment on the eastern flank of the Emperor Seamounts in the far north-west Pacific, is a unique location to test for deep ocean circulation changes. Results from Deep Sea Drilling Project (DSDP) Site 192 showed that sedimentation

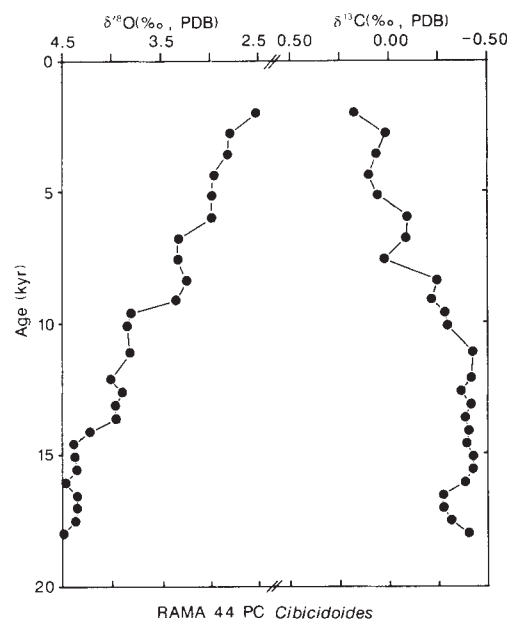


Fig. 1 Oxygen and carbon isotope results (‰, PDB) on the benthic foraminifera *Cibicidoides* from Meiji Tongue, in the far north-west Pacific. Oxygen isotope results show a typical pattern due to deglaciation of land masses, which is used to date this sediment sequence (see text). Carbon isotope results (plotted on an expanded x axis) reveal the typical glacial-interglacial change in seawater chemistry, with no evidence of nutrient depletion at 3 km water depth during glacial time.

Table 1 Stable isotope results on *Cibicidoides* spp. (>150 μm) from core RAMA 44P

Depth (cm)	$\delta^{18}\text{O}$ (‰, PDB)	$\delta^{13}\text{C}$ (‰, PDB)
6	2.53	0.17
11	2.80	0.01
16	2.82	0.06
21	2.96	0.10
26	2.98	0.05
31	2.98	-0.10
36	3.32	-0.09
41	3.33	0.02
46	3.24	-0.25
51	3.35	-0.22
56	3.80	-0.29
61	3.84	-0.30
71	3.81	-0.43
81	4.01	-0.42
86	3.89	-0.37
91	3.96	-0.42
96	3.95	-0.39
101	4.22	-0.41
106	4.39	-0.40
111	4.37	-0.43
116	4.35	-0.43
121	4.47	-0.39
126	4.35	-0.27
131	4.35	-0.28
136	4.37	-0.32
141	4.49	-0.41

rates are high (>5 cm per 10^3 yr) and foraminifera are preserved in the sediment¹⁶. Mammertx¹⁷ presented evidence that sedimentation on Meiji Tongue is current-controlled, and by analogy with abyssal sediment drifts in the North Atlantic, she suggested southward thermohaline flow transported the fine-grained sediment. Piston core RAMA 44 was taken on Scripps Institution of Oceanography Rama Expedition at the crest of Meiji Seamount, the same location as DSDP 192 (53° N, 164° 39.2' E, 2,980 m). The upper 1.5 m of this core is silty diatomaceous clay with varying carbonate, ice rafted debris and volcanic ash content. Benthic foraminifera are common and well-preserved in the upper 140 cm of RAMA 44P, and specimens of *Cibicidoides wuellerstorfi* and *C. kullenbergi* >150 μm were picked for stable isotope analysis. Analyses were done in the laboratory of Dr R. G. Fairbanks at Lamont-Doherty Geological Observatory on a Finnegan MAT 251 mass spectrometer. Details of the history of sediment accumulation and faunal change at RAMA 44P will be the subject of a later paper.

Oxygen isotope results show a glacial to interglacial pattern typical of cores with high rates of sedimentation (Table 1; Fig. 1). Maximum enrichment in ^{18}O , indicating maximum volume of continental ice and minimum deep sea temperatures, is found below ~130 cm and the uppermost abrupt decrease in values at 50 cm marks the beginning of the Holocene. Carbon isotope ratios in glacial samples are ~0.5‰ lower than interglacial samples, close to the estimated glacial-interglacial change in seawater carbon isotopic composition⁸. By comparison with a very similar record of *Cibicidoides* $\delta^{18}\text{O}$ from the North Atlantic¹⁸, the two steps in continental deglaciation are recognized at 101 and 51 cm at RAMA 44P. These events, which have not been recognized previously in the Pacific, have been dated in the Atlantic at ~14 and 9 kyr BP using the accelerator radiocarbon technique¹⁹. Using these events to date the stable isotope record of RAMA 44P, and assuming a core-top age of 1 kyr and a date of 18 kyr for 140 cm, results are plotted against age in Fig. 1.

Glacial-interglacial changes in Pacific seawater composition can be illustrated best by comparing results from two widely

spaced locations. Latest Quaternary *Cibicidoides* data from eastern equatorial Pacific core KNR 73, 3PC (0° 22'S, 106° 11'W, 3,606 m; ref. 8) reveal the same pattern of change as found at RAMA 44P (Fig. 2). Slightly lower $\delta^{18}\text{O}$ values and slightly higher $\delta^{13}\text{C}$ values in KNR73-3PC samples of glacial age compared to RAMA 44P probably result from the influence of bioturbation on the former core, which has a lower rate of sedimentation. Glacial $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ results on *Cibicidoides* at RAMA 44P are virtually identical with those from east equatorial Pacific core TRI63-31B (3° 37.2'S, 83° 58.4'W, 3,200 m; Curry *et al.*, in preparation). Thus, if there were significant temperature and nutrient differences between the far northwestern and equatorial Pacific during glacial time, they are not evident using the oxygen isotope method in these cores.

The 0.5‰ depletion in seawater ^{13}C during glacial times is thought to result from a decrease in continental biomass owing to the presence of ice sheets, as originally proposed by Shackleton²⁰; from increased continental aridity²¹; and from the release, during times of lowered sea level, of organic matter stored on continental shelves²². Because *Cibicidoides* species are the best proxy for seawater carbon isotopic composition^{23,24}, and as $\delta^{13}\text{C}$ of seawater is closely linked to seawater nutrient and oxygen levels²⁵, this benthic foraminiferal genus is frequently used to monitor oceanic palaeochemistry. As an example, the 1.0‰ decrease in deep Pacific benthic foraminiferal $\delta^{13}\text{C}$ compared to deep Atlantic benthic foraminiferal $\delta^{13}\text{C}$ reflects the 1.4 $\mu\text{M kg}^{-1}$ increase in deep ocean PO_4 and the 215 $\mu\text{M kg}^{-1}$ decrease in deep ocean O_2 . The glacial decrease in $\delta^{13}\text{C}$ in the far north-west Pacific (Figs 1 and 2) simply reflects the global change in seawater composition; there is no evidence for nutrient-depletion at 3 km.

It has been known for some time that the glacial-interglacial carbon isotope patterns from the eastern, central and western equatorial Pacific^{6,8,26} are essentially the same. This uniformity has been used to argue that the Pacific Ocean was as chemically homogeneous during glaciation as it is today⁸, but without a suitable sediment core from high northern latitudes a considerable part of the Pacific was not represented. The RAMA 44P results extend the range of chemically uniform water to 53°N, but this core sampled the ocean at only one depth (3 km). It will be important to core the far north-west Pacific from the crests of seamounts to the axis of thickest accumulation on Meiji Tongue at 4 km (ref. 27) to determine the extent of palaeo-hydrographic change over a large depth range in a small geographic area.

No high-quality stable isotope record of benthic foraminifera from the north-east Pacific is yet available, but a few stable isotope analyses suggest the same pattern as seen elsewhere in the Pacific. A core taken at ocean station Papa (50° N, 145° S, 4,300 m), was provided by S. Manganini and S. Honjo. Specimens of the dissolution resistant benthic foraminiferal species *N. umbonifera* which were examined from two samples at the core top had $\delta^{18}\text{O} = 2.86 \pm 0.02\text{‰}$ and $\delta^{13}\text{C} = -0.34 \pm 0.20\text{‰}$, and two samples from a deeper interval relatively high in carbonate content had $\delta^{18}\text{O} = 4.29 \pm 0.06\text{‰}$, and $\delta^{13}\text{C} = -0.61 \pm 0.01\text{‰}$. The range in $\delta^{18}\text{O}$ suggests these samples are glacial and interglacial pairs, but a series of data is necessary to identify with confidence the true glacial maximum. Specimens of *C. wuellerstorfi*, found only in the deep samples, had $\delta^{18}\text{O} = 3.94 \pm 0.01\text{‰}$, and $\delta^{13}\text{C} = -0.30 \pm 0.08\text{‰}$. These values for *C. wuellerstorfi* fall on the $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ trend defined by the RAMA 44P and KNR73-3PC data (Fig. 1), which suggests that during glacial time the deep north-east, north-west, and equatorial Pacific all had about the same carbon isotope chemistry and nutrient chemistry.

Glacial-interglacial comparisons of geochemical data between ocean basins have only just begun (refs 6, 8, 13, and W. B. Curry *et al.*, in preparation), and are the best method for studying deep ocean circulation patterns. Even with the results of RAMA 44P, the deep North Pacific is still under-represented. Carbon isotope ratios of late glacial age in the deep North

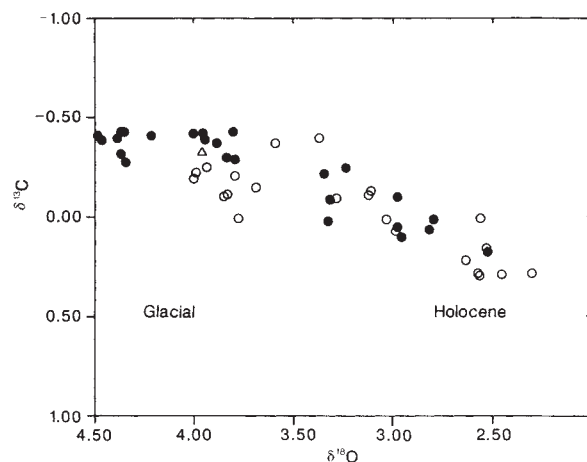


Fig. 2 Plot of oxygen and carbon isotope results on the Pacific Ocean benthic foraminifera *Cibicidoides*. Data from the far north-west Pacific (core RAMA 44P) are plotted as solid circles, equatorial Pacific data (core KNR73, 3PC) are plotted as open circles, and the one north-east Pacific sample (station Papa core) is plotted as an open triangle. The overall linear pattern reflects the uniformly changing seawater chemistry of the deep equatorial and North Pacific from glacial (high $\delta^{18}\text{O}$) to interglacial (low $\delta^{18}\text{O}$) time.

Atlantic (at 4.5 km (ref. 10)) are at times lower than those observed in the north-west Pacific. If glacial $\delta^{13}\text{C}$ is found to be invariant below 2.5 km in well-dated cores from the North Pacific, as the results of J.-C. Duplessy *et al.* (in preparation) suggest, then we will have to identify the source of nutrient depletion. It may turn out that the nutrient-depleted intermediate waters flowing southward at depths less than 2.5 km mixed with underlying northward-flowing nutrient-rich waters of southern origin during glacial times. Regardless, it will be important to check the reliability of conclusions based on $\delta^{13}\text{C}$ data with additional proxies for ocean nutrient content and current direction.

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Global surface-temperature responses to major volcanic eruptions

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The impacts of pollution resulting from large explosive volcanic eruptions on the atmospheric heat budget and planetary albedo are not in doubt^{1,2}. However, the effects of volcanic aerosols on the surface climate are less clear and still controversial^{3,4}. In a previous study⁵ it was shown that significant surface cooling occurs over the landmasses of the Northern Hemisphere in the first few months after a major eruption in that hemisphere. Here we extend that work using new surface-air temperature compilations based on land and marine data⁶ for both the Northern and Southern Hemispheres. Our results indicate that major Northern Hemisphere eruptions have an immediate effect on the Northern Hemisphere average surface temperature but little or no effect on the Southern Hemisphere average. Southern Hemisphere eruptions affect both Southern and Northern Hemisphere temperatures after a lag of between six months and a year.

Empirical studies have indicated that major explosive eruptions are followed by a decrease in seasonal or annual surface temperatures of up to a few tenths of a degree^{3,7,8}. Whilst consistent with model simulations⁹⁻¹¹, this cooling is near the level of natural variability, leaving room for doubt as to the reality of the effect; the volcanic signal in surface-air temperature records tends to be hidden within the noise of climatic variability caused by other factors^{12,13}. Superposed epoch analysis (SEA)^{14,15} has been widely used in studies of the effects of volcanoes on climate^{3,7} in order to reduce this noise level. Averaging across a set of volcanic events maximizes the common features of the response: the volcanic signal. We have used the same major volcanic events (Table 1) as previously⁵, but the temperature compilations used here are more representative of hemispheric and global temperatures than previous series, as they include more than Northern Hemisphere land-based data⁶. The sea surface and marine air temperatures in this compilation were corrected to remove the effects of non-climatic factors. The relative shortness of the record for the Southern Ocean and Antarctica^{6,16} means that the Southern Hemisphere compilation

Table 1 Volcanic events included in the superposed epoch analysis

Northern Hemisphere eruptions		
Date (yr, month)	Event	Position
1902, 5	Pelé	14.8°N 61.2°W
1902, 5	Soufrière	13.4°N 61.2°W
1907, 3	Ksudach	51.8°N 157.5°E
1912, 6	Novarupta (Katmai)	58.3°N 155.2°W
1956, 3	Bezymianny	57.1°N 160.7°E
Southern Hemisphere eruptions		
Date (yr, month)	Event	Position
1883, 8	Krakatau	6.1°S 105.4°E
1886, 6	Tarawera	38.2°S 176.5°E
1932, 4	Azul (Quizapu)	35.7°S 70.8°W
1963, 3	Agung	8.3°S 115.5°E

These data are abridged from ref. 5. Selection based on geological²⁵ and other evidence²⁶⁻²⁸. Note, the eruption sequence of El Chichón in 1982 rivals these in significance but has not been included in the analysis as sufficient temperature data are not yet available for the post-eruption period. Interestingly, a study of what data are available indicates no marked effect on surface-air temperature, despite the undoubted large scale of the atmospheric injection.

is generally representative of at least 50% of the hemisphere before 1957.

The date of each eruption event was defined as a 'key date': month zero. To prevent bias which may result from greater temperature variability in the winter half-year, the temperature data were standardized by subtracting the appropriate long-term mean from each monthly temperature and then dividing by the relevant monthly standard deviation (s.d.) (Table 2). The resulting temperature series were then expressed as departures from prevailing temperature levels and composited for months -24 to +48 around the key dates. In order to minimize any seasonal bias caused by the correction of the temperature data on an annual basis⁶, the prevailing temperature level was defined for each month separately as the relevant monthly mean for the five years preceding month zero. This differs from the definition of the prevailing temperature level used previously⁵. A Monte Carlo technique^{5,14} was used to test the statistical significance of the results. A total of 500 composite temperature series were generated using four randomly selected month zeros. The distribution of the resulting 500 values for months chosen at random from +1 to +48 was used to estimate significance levels. A one-tailed test was used because it is generally accepted that volcanic eruptions do not cause large-scale surface warming³.

Table 2 Monthly standard deviations (K) of mean land/marine surface-air temperatures⁶

Month	Standard deviation (K)		
	Northern Hemisphere	Southern Hemisphere	Global
January	0.39	0.22	0.27
February	0.36	0.21	0.26
March	0.27	0.21	0.21
April	0.23	0.21	0.20
May	0.22	0.25	0.20
June	0.19	0.24	0.18
July	0.20	0.24	0.18
August	0.20	0.22	0.17
September	0.20	0.20	0.17
October	0.26	0.20	0.20
November	0.32	0.20	0.24
December	0.36	0.20	0.24

The reference period is 1861 to 1979.

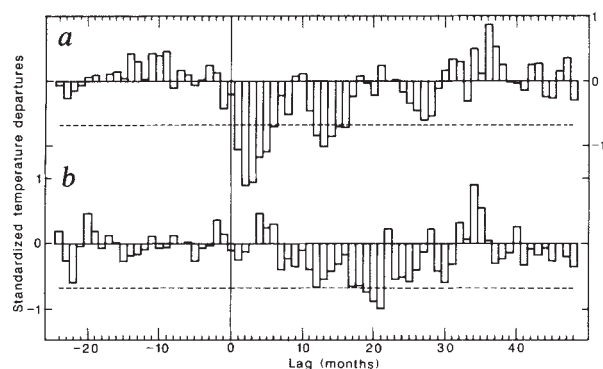


Fig. 1 Superposed epoch analysis of standardized monthly Northern Hemisphere surface-air temperatures for: *a*, the Northern Hemisphere eruption sample shown in Table 1; and *b*, the Southern Hemisphere eruption sample shown in Table 1. To convert a standardized departure to an actual temperature departure, multiply by the appropriate monthly standard deviation in Table 2. For negative departures the 0.05 significance level based on Monte Carlo simulations is shown by the dashed line at -0.68 s.d.

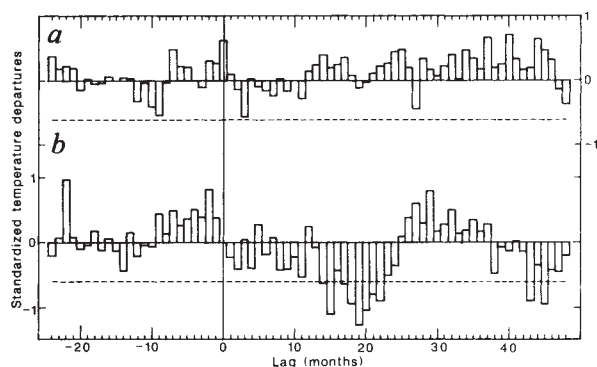


Fig. 2 Superposed epoch analysis of standardized monthly Southern Hemisphere surface-air temperature series using: *a*, the Northern Hemisphere eruption sample shown in Table 1; and *b*, the Southern Hemisphere eruption sample shown in Table 1. For negative departures the 0.05 significance level based on Monte Carlo simulations is shown by the straight line at -0.61 s.d.

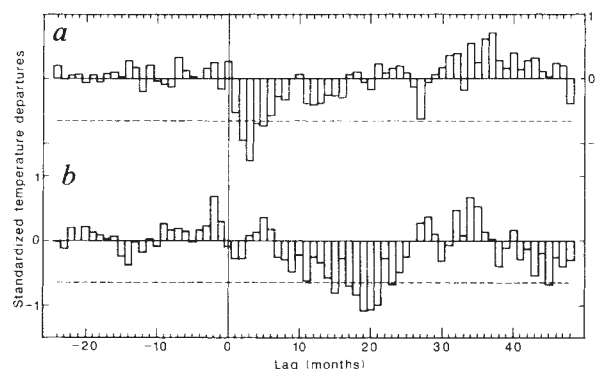


Fig. 3 Superposed epoch analysis of standardized monthly global surface-air temperature series using: *a*, the Northern Hemisphere eruption sample shown in Table 1; and *b*, the Southern Hemisphere eruption sample shown in Table 1. For negative departures the 0.05 significance levels based on Monte Carlo simulations is shown by the straight line at -0.65 s.d.

Figure 1a shows the results of the SEA for the Northern Hemisphere eruption sample. As P.M.K. and C.B.S. have demonstrated previously⁵, the response of Northern Hemisphere surface temperatures to northern eruptions is very rapid. A decrease in temperature soon follows month zero, with a maximum temperature drop of -1.60 s.d. (typically 0.32 K in summer) in the second month after the eruption date (month +2). Such a rapid response is surprising in view of the relatively slow meridional spread of an aerosol and the ocean's thermal inertia¹⁷. Indeed, some modelling results point to a delayed response in averaged surface temperature^{9,10} for these reasons but these studies were simulations of the effects of low-altitude eruptions. A rapid response to extra-tropical eruptions of less than six months has been suggested by other modelling work¹¹.

The temperature departures remain negative for much of the first two years following an eruption with a secondary minimum of -1.00 s.d. in month +13. Another minimum occurs at the beginning of the third year after the eruption. Temperatures then return to pre-eruption levels. All the northern key dates fell between March and June. Thus, a seasonal cycle is suggested with enhanced response in the summer and little, if any, effect in the winter months. It is likely that this seasonality is a combination of the effects of latitudinal and seasonal variation in radiation budget^{2,11} and mixed layer depth changes¹⁸. Also possible is a seasonally dependent response through the Hadley circulation⁹ and/or atmosphere-cryosphere interactions, which may be greatest in the spring and autumn¹⁰.

The Northern Hemisphere response to Southern Hemisphere eruptions (Fig. 1b) is not as marked and occurs after a lag of a few months. Negative temperature anomalies occur after month +6 and persist (in all but three months) for a further two years, a remarkable sequence. However, significant departures are not seen until the second year after month zero and the maximum departure (-1.00 s.d.) occurs in month +21. A return to prevailing temperature levels occurs in the first half of year three.

We now consider the response of Southern Hemisphere surface-air temperature. Lack of data has prevented such an analysis in the past. Interestingly, Southern Hemisphere temperatures do not appear to respond significantly to eruptions in the Northern Hemisphere (Fig. 2a). This was unexpected because certain modelling results imply that there should be a marked response^{9,10}. It is likely that the slower response time of the 'Ocean Hemisphere' is the explanation for this. The limited forcing caused by aerosols crossing the equator is insufficient to elicit a significant response given the thermal inertia of the southern oceans and obfuscation by noise.

Figure 2b indicates a marked response in Southern Hemisphere temperatures to an aerosol load originating in the Northern Hemisphere. There is a suggestion of immediate cooling after the eruption key date but not until month +13 does the temperature sequence show a series of negative anomalies. The major effect of Southern Hemisphere eruptions is seen in the second year after month zero when eight months show significant negative temperature departures with a maximum of -1.27 s.d. in month +19. This delay is consistent with the slow response time of the dominant oceans of the hemisphere.

Finally, we consider the response of globally averaged surface temperatures⁶ to major eruptions in the Northern and Southern Hemispheres. Not surprisingly, the composites combine the features of the hemispheric analyses. Note the rapid response of global temperature to Northern Hemisphere eruptions (Fig. 3a) and the delayed response to Southern Hemisphere events (Fig. 3b). The largest negative temperature departures are -1.27 s.d. for northern eruptions (typically 0.3 K) in month +3 and for southern eruptions, -1.09 s.d. in month +19.

The magnitudes of the response in the surface temperatures of both hemispheres to stratospheric aerosol loading in the same hemisphere are approximately the same. Maximum cooling is between -1.25 and -1.60 s.d. with the peak effects, once evident, lasting for about six months. In our view, the difference in response time is a result of the different proportions of land and ocean in the two hemispheres. The major characteristics of the composite responses seen in Figs 1, 2 and 3 are clear in most of the individual eruption sequences, although they are contaminated by noise (see, for example, the sequences shown by P.M.K. and C.B.S.⁵). Empirical analyses cannot prove a physical link between volcanic eruptions and surface climate but the statistical evidence strongly suggests such a link.

There are many questions that remain unanswered. In particular, the radiative and dynamical mechanisms which cause surface cooling following an injection of volcanic aerosol into the stratosphere require elucidation and interactions with other short timescale climatic phenomena must be considered. For example, the Southern Oscillation/El Niño (ENSO) phenomenon operates on a similar timescale and has a significant effect on largescale temperatures¹⁹. After the eruptions of El Chichón, stratospheric aerosol spread widely²⁰⁻²² and caused significant warming in the stratosphere^{23,24}. It has been claimed that the strong ENSO event of 1982/83 obscured the tropospheric effects of El Chichón^{3,13}. Nevertheless the lack of any clear evidence of surface-air temperature response to that event warrants further careful study.

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Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment

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A series of long-chain (C_{37} , C_{38} , C_{39}), primarily di- and tri-unsaturated methyl and ethyl ketones, first identified in sediments from Walvis Ridge off West Africa and from the Black Sea¹, has been found in marine sediments throughout the world². The marine coccolithophorid *Emiliania huxleyi* and members of the class *Prymnesiophyceae* are now the recognized sources of these compounds^{3,4}. Experiments with laboratory cultures of algae showed the degree of unsaturation in the ketone series biosynthesized depends on growth temperature^{2,5}, a physiological response observed for classical membrane lipids⁶. Brassell and co-workers^{2,7} thus proposed that systematic fluctuations in the unsaturation of these alkenones noted down-core in sediments from the Kane Gap region of the north-east tropical Atlantic Ocean and correlated with glacial-interglacial cycles provide an organic geochemical measure of past sea-surface water temperatures. Using laboratory cultures of *E. huxleyi*, we have calibrated changes in the unsaturation pattern of the long-chain ketone series versus growth temperature. The calibration curve is linear and accurately predicts unsaturation patterns observed in natural particulate materials collected from oceanic waters of known temperature. We present evidence supporting the proposed palaeotemperature hypothesis^{2,7} and suggesting absolute 'sea-surface temperatures' for a given oceanic location can be estimated from an analysis of long-chain ketone compositions preserved in glacial and interglacial horizons of deep-sea sediment cores.

Batch cultures of *Emiliania huxleyi* were grown at five temperatures (8, 10, 15, 20 and 25°C) and analysed by gas chromatography for long-chain ketone composition. Systematic compositional differences were noted between growth temperatures (8, 10, 15, 20 and 25°C) and analysed by gas obtained for cells grown at two extremes of temperature, 10°C and 25°C, illustrates the extent of the qualitative differences observed. From the chromatographic data, the degree of unsaturation for ketones with 37 carbon atoms was quantified for cells grown at each temperature using an unsaturation index

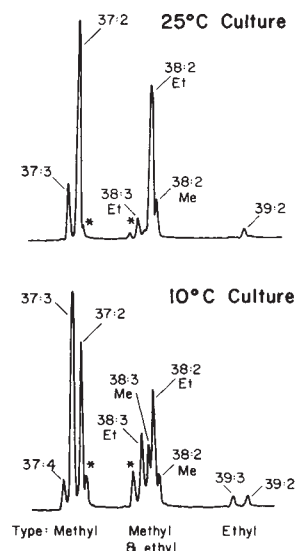


Fig. 1 Gas chromatograms of the long-chain, unsaturated ketone compositions measured in cultures of *E. huxleyi* grown at 10°C and 25°C. Individual compounds are identified by carbon chain length:number of double bonds. Methyl ketones have C_{37} and C_{38} chain lengths; ethyl ketones have C_{38} and C_{39} chain lengths. Overlapping methyl (Me) and ethyl (Et) ketones with C_{38} chain lengths are indicated. The peaks marked with an asterisk are di-unsaturated fatty acids ($n-C_{36}$) naturally produced by this plant as a methyl and ethyl ester⁴.

Methods. Batches of cells from a single strain of *E. huxleyi* (Northeast Pacific Culture Collection, University of British Columbia, Vancouver, British Columbia) were grown at five different constant temperatures under sterile conditions using filtered natural seawater enhanced with an artificial growth medium (549 μM NO_3^- , 21.8 μM glycerol- PO_4^{2-} , 14.9 μM EDTA, 61.5 μM H_3BO_3 , 6.0 μM $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 0.60 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 2.4 μM $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.25 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 μM $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 μM thiamine, 0.0015 μM vitamin B_{12} and 0.0041 μM biotin). A 14:10 light/dark cycle (250-300 $\mu\text{E m}^{-2} \text{s}^{-1}$) was used in all cases. At a logarithmic stage of growth (monitored by *in vivo* fluorescence), cell samples (10^5 to 10^6 cells) were harvested from each batch for subsequent lipid analysis. Cell samples, stored frozen on GF/C filters until analysis, were extracted ultrasonically in 50% hexane and acetone. The extracts were fractionated into hexane, the hexane was removed by rotary evaporation and the organic residue was chromatographed on a short bed of silica gel (1.4 g, Kiesegel 60 70-230 mesh). The fraction containing the long-chain, unsaturated ketones was analysed by capillary gas chromatography (on-column injection; 30 m $\times$ 0.25 mm i.d. DB-5 fused silica column, J & W Scientific) operated with hydrogen as a carrier gas (10 p.s.i. column head pressure) and temperature programming (100 to 300°C at 5°C min⁻¹); flame ionization detection.

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Table 1 Unsaturation indexes measured in *E. huxleyi* grown in batch culture at different temperatures and harvested at a logarithmic stage of growth

Temperature	$U_{37}^{K*†}$	$U_{37}^{K†}$	$U_{37}^{K‡}$	n
5	0.00–0.11	—	—	—
8	—	0.17	0.29	1
10	—	0.31 ± 0.017	0.38 ± 0.027	4
15	0.17, 0.20 0.26, 0.34	0.54 ± 0.016	0.55 ± 0.014	4
20	0.40	0.74 ± 0.017	0.74 ± 0.017	7
25	0.73	0.86 ± 0.017	0.86 ± 0.017	4

* Data extrapolated from ref. 2.

† $U_{37}^{K*} = [37:2 - 37:4]/[37:2 + 37:3 + 37:4]$.‡ $U_{37}^{K‡} = [37:2]/[37:2 + 37:3]$.

$U_{37}^{K*} = [37:2 - 37:4]/[37:2 + 37:3 + 37:4]$, originally defined by Brassell *et al.*². In the formula, 37:2, 37:3 and 37:4 represent concentrations of di-, tri- and tetra-unsaturated ketones with 37 carbon atoms, respectively, measured in any given sample. Average values of this index derived from analyses of replicate samples of the plant grown at each temperature are given in Table 1.

Values of U_{37}^{K*} show the clear increase with growth temperature noted in the previous study of this plant². However, specific details of our results differ from the earlier findings in two fundamental respects. First, our values of U_{37}^{K*} for a given growth temperature are consistently higher; values of U_{37}^{K*} from the earlier work are included in Table 1 for comparison. The cause of this apparent discrepancy is uncertain and warrants further laboratory investigation. Systematic study of other strains of *E. huxleyi* will reveal the extent to which values of U_{37}^{K*} measured for a given temperature depend on the algal clone under investigation. Other culture conditions, such as the nutrient status and growth rate of the cell, could also affect unsaturation patterns in the ketone composition. Values of U_{37}^{K*} measured in senescent cells (0.34 at 10°C, 0.74 at 20°C and 0.83 at 25°C) showed little difference from those in cells harvested at a healthy, logarithmic stage of growth (Table 1), suggesting these other culture conditions have only secondary importance in determining the degree of ketone unsaturation. Second, we found that values of U_{37}^{K*} measured in different batches of the plant grown at a given temperature are much more reproducible than was previously reported. The total variance in measurements of this parameter for replicate samples was ± 0.02 units in our study. The absolute magnitude of this uncertainty seems to be independent of the cell growth temperature and includes the combined effects of batch-to-batch culture variability and of analytical imprecision.

The values of U_{37}^{K*} measured in our cultures covary linearly with growth temperature. A least squares fit of the data yields the line $U_{37}^{K*} = 0.040T - 0.11$ ($r = 0.989$) and provides a calibration for the organic 'thermometer' biosynthesized by *E. huxleyi*. The slope defines the magnitude that U_{37}^{K*} will change for a 1°C change in growth temperature; that is, 0.040 units per °C. As the unsaturation index is measurable to ± 0.02 units, growth temperatures predicted from this calibration curve are accurate potentially to $\pm 0.5^\circ\text{C}$.

Because the tetra-unsaturated ketone (37:4) is rarely detected in sediments², it may be omitted from the definition of U_{37}^{K*} . Values of the unsaturation index were recalculated using the simplified expression: $U_{37}^{K*} = [37:2]/[37:2 + 37:3]$. Comparison of these new values with those calculated by the original definition (Table 1) shows that 37:4 affects the index only when cells are grown at temperatures $< 15^\circ\text{C}$. The difference between values calculated by these two definitions of U_{37}^{K*} then increases steadily as growth temperature drops lower. U_{37}^{K*} values, calculated by the simplified expression, were regressed linearly versus

temperature to yield another line ($U_{37}^{K*} = 0.033T + 0.043$, $r = 0.997$). The slope of this line is flatter than that obtained in the previous analysis (0.040) and indicates that a 0.033 unit change in U_{37}^{K*} will occur for every 1°C change in the growth temperature of the cell.

A test was conducted to determine if the temperature calibration for U_{37}^{K*} , based on our laboratory cultures of *E. huxleyi*, is upheld by natural populations of phytoplankton growing in the ocean. Samples of suspended particulate material (SPM) from surface waters in a variety of oceanic locations were analysed for long-chain, unsaturated ketone composition. Values of U_{37}^{K*} , paired with the water temperature measured in the surface mixed layer (SML) at each sampling location, are given in Table 2. The U_{37}^{K*} value and SML temperature for the mixed plankton sample from Cape Finisterre, included in Table 2, are previously reported data².

Figure 2 shows U_{37}^{K*} values measured in the SPM samples (solid circles) plotted against SML temperature. The dashed line represents the calibration line ($U_{37}^{K*} = 0.033T + 0.043$) obtained from the laboratory cultures of *E. huxleyi*. Close agreement between field and laboratory data is evident and suggests either: (1) *E. huxleyi* is the dominant species setting the value of U_{37}^{K*} measured in the field samples; or (2) other species of algae, related to *E. huxleyi* and also capable of biosynthesizing long-chain, unsaturated ketones, have the same biochemical response to growth temperature.

We cannot formally evaluate the importance of these possibilities at present. However, support for the first possibility comes from the biogeography of marine coccolithophorids in the ocean⁸. In the Pacific, *E. huxleyi* is a ubiquitous inhabitant

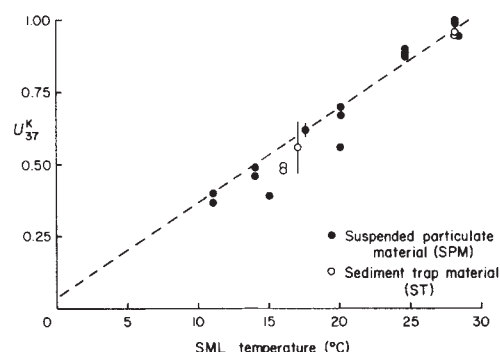
Table 2 The unsaturation index in suspended particulate material and in vertically transported particulate material from various locations in the ocean

Site	Coordinates	Sample type (collection depth)	U_{37}^{K*}	SML temperature (°C)
Cariaco Trench	10°22' N 65°18' W	SPM (50 m)	0.90	24.5
		SPM (150 m)	0.89	
		SPM (250 m)	0.88	
Peru	15°10' S 75°30' W	SPM (0–35 m)	0.62 ± 0.025 (n = 6)	17.5
		ST (14 and 53 m)	0.56 ± 0.09 (n = 15)	
		ST (100 m)	0.48	
VERTEX I	35°50' N 124°40' W	ST (750 m)	0.50	16
VERTEX II	18°00' N 108°00' W	SPM (5 m)	1.0	28
		SPM (200 m)	0.99	
		ST (100 m)	0.96	
VERTEX III	15°47' N 108°18' W	ST (470 m)	0.95	28
		ST (1500 m)	0.96	
		SPM (5 m)	1.0	
		SPM (200 m)	0.95	
		ST (100 m)	0.96	
VERTEX VA	33°10' N 138°37' W	ST (470 m)	0.95	20
		ST (1500 m)	0.96	
		SPM (10 m)	0.67	
		SPM (100 m)	0.70	
VERTEX VB	35°22' N 126°10' W	SPM (10 m)	0.56	14
		SPM (10 m)	0.49	
VERTEX VX	35°31' N 124°59' W	SPM (10 m)	0.39	15
		SPM (10 m)	0.40	
VERTEX VC	36°06' N 122°38' W	SPM (10 m)	0.37	11
		SPM (60 m)	0.37	
PARFLUX E	13°30' N 54°00' W	ST (389 m)	0.95	*
PARFLUX P ₁	15°21' N 151°28' W	ST (5,068 m)	0.94	*
		ST (389 m)	0.93	
		ST (5,582 m)	0.95	
Cape Finisterre	43°11' N 09°33' W	—	0.46	14

Saturation index, ($U_{37}^{K*} = [37:2]/[37:2 + 37:3]$). SPM, suspended particulate material; ST, vertically transported particulate material; SML, surface mixed layer.

* Hydrographic data not available for the long period of sediment trap deployment.

Fig. 2 Plot of the unsaturation index, $U_{37}^K = [37:2]/[37:2 + 37:3]$, against surface mixed layer (SML) temperature for natural particulate samples collected at various geographic locations throughout the world's oceans (Table 2). Solid circles represent data for suspended particulate material (SPM) collected using water bottles or *in situ* pumps; open circles represent data for vertically transported particulate material (ST) collected in sediment traps. The dashed line represents the temperature calibration curve ($U_{37}^K = 0.033T + 0.043$; $r = 0.997$) determined for cultures of *E. huxleyi* grown under laboratory conditions.



of waters extending from the tropics to the subarctic. It is the dominant coccolithophorid found in waters cooler than 20°C (transitional to subarctic regions) and warmer than 25°C (tropical regions). Thus, at least for much of the contemporary ocean, *E. huxleyi* very likely represents a major source of the long-chain, unsaturated ketone signal deposited in sediments. *E. huxleyi*, on the other hand, coexists with a diversity of other related species in waters of intermediate temperatures (20–25°C, temperate regions). Regarding the second possibility, laboratory calibration of cultures of other genera of prymnesiophytes should provide sufficient information in the future to evaluate what effect a more complex community of plants, all potentially capable of producing long-chain, unsaturated ketones, may have on values of U_{37}^K measured in natural particulate material from temperate regions.

If U_{37}^K values set by algal sources living in surface waters are not significantly altered during sedimentation, stratigraphic records of long-chain ketone unsaturation with depth in sediment cores will readily translate into absolute palaeotemperature curves using the temperature calibration for this index. Sinking particulate material captured in sediment traps deployed at various depths (ST in Table 2) was available to evaluate the behaviour of U_{37}^K in the sedimentation process. U_{37}^K values measured in sediment trap materials showed no consistent change with collection depth or significant difference from the values measured in SPM from overlying waters (Table 2). These data compare samples separated vertically in space by hundreds to thousands of metres water depth.

The long-chain ketones measured in sinking particles almost certainly derive through sedimentation from algae living in the overlying photic zone. U_{37}^K values for each trap were thus assigned the temperature of the corresponding overlying SML (Table 2). U_{37}^K values and SML temperatures for trap samples are plotted as open circles in Fig. 2. The sediment trap data generally fall along the calibration line defined by laboratory cultures of *E. huxleyi*. These observations support the suggestion² that once the algae, living in the photic zone, set the value of U_{37}^K , this organic record of water temperature is not appreciably changed by sedimentation.

A least squares analysis of all of the field data, including SPM and sediment trap samples, yields the line $U_{37}^K = 0.037T - 0.07$ ($r = 0.983$, $n = 25$). This line is remarkably similar to the calibration line obtained from *E. huxleyi* grown in the laboratory. Further field testing is necessary to assess whether the difference in the slope and intercept of the field and laboratory calibration lines are significant.

Values of U_{37}^K recorded in sediments from Kane Gap in the north-east tropical Atlantic have increased by 0.12 units since the last glacial maximum, 18,000 yr BP². Using the slope of the laboratory calibration line (0.033 units per °C), the 0.12 unit increase in U_{37}^K corresponds to a change of 3.6°C in the seasonally averaged mean SML temperature at this location. This prediction is consistent with the change in surface water temperature estimated for this region of the Atlantic from analyses

of oxygen isotopes in foraminiferal assemblages⁹. Values of U_{37}^K in sediments from Kane Gap are reported as 0.89 at present and 0.78 at the last glacial maximum². Using the equation that calibrates U_{37}^K versus temperature, these values correspond to absolute temperatures of 25.7°C and 22.3°C, respectively. These temperatures would represent the mean annual sea-surface temperature at this location now and 18,000 yr BP. Surface waters at this oceanic location remain at ~26°C throughout the year², in excellent agreement with the organic geochemical assessment. The organic geochemical prediction for the last glacial maximum also lies nicely between the 22°C and 23°C isotherms on the map of mean annual sea-surface temperatures for the Atlantic 18,000 yr BP⁹.

Answers to many questions about this novel series of organic compounds are needed before the potential and limitations of this new palaeoenvironmental tool can be fully recognized. In particular, is the value of U_{37}^K , preserved in sediments, actually measuring 'sea-surface temperature' at all geographical locations? The algae that set the value of this geochemical index may not reside at the surface of the photic zone in all geographical regions but rather, in particular oceanic regimes, deeper in this layer, possibly in colder water. The vertical structure of plant communities in the ocean certainly varies from region to region in response to geographical differences in physical oceanography. How far back in the geological record do long-chain, unsaturated ketones store palaeotemperature information^{2,7}? And, why are the long-chain, unsaturated ketones preserved in sediments at all? Most unsaturated lipids of phytoplanktonic origin do not survive passage through the pelagic food chain¹⁰ and hence, never even enter the sedimentary record. Long carbon chain length, unusual double bond positions¹ and possibly molecular geometry provide at least partial explanations for the preservation of these intriguing biological markers.

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Long-term effects of organic and conventional farming on soil erosion

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Conventional, intensive tillage farming systems have greatly increased crop production and labour efficiency. But, serious questions are being raised about the energy-intensive nature of these systems and their adverse effects on soil productivity and environmental quality^{1,2}. This concern has led to an increasing interest in organic farming systems because they may reduce some of the negative effects of conventional agriculture on the environment^{3,4}. We compare the long-term effects (since 1948) of organic and conventional farming on selected properties of the same soil. The organically-farmed soil had significantly higher organic matter content, thicker topsoil depth, higher polysaccharide content, lower modulus of rupture and less soil erosion than the conventionally-farmed soil. This study indicates that, in the long term, the organic farming system was more effective than the conventional farming system in reducing soil erosion and, therefore, in maintaining soil productivity.

Organic farming differs from conventional farming mainly in tillage methods, crop rotations, fertilizer applications, and pest control methods. Whereas conventional farming systems depend on chemical fertilizers and pesticides, organic farming systems avoid or largely exclude their use by relying upon crop rotations, manuring, mechanical cultivation, organic fertilizers, and biological pest control to maintain soil productivity, supply plant nutrients, and control pests⁵.

We studied two adjacent winter wheat (*Triticum aestivum*) fields from two farms, one organically managed and the other conventionally managed, containing a study area where all soil-forming factors as described by Jenny⁶, except management, were equal. The study area was made up of Naff silt loam (fine-silty, mixed, mesic Ultic Argixeroll), a dark-coloured, well-drained soil which formed under grass in deep deposits of loess mixed with some thin layers of volcanic ash⁷. Typically, Naff soils have a silt loam surface A1 horizon 20–46 cm thick, overlying an A2 or AB horizon of heavy silt loam. The next layer is a strong, silty clay loam Bt or argillic B horizon (a layer of clay accumulation that is significantly denser than the layer(s) above). The slope of the study area was ~6.5%, but ranged from 5 to 8%.

The two farms were situated 30 km south of Spokane, Washington, USA, in the northern part of the productive dryland winter wheat area referred to as the Palouse. The organic farm was a 320 ha wheat farm identified in the US Department of Agriculture report on organic farming⁵ as a farm which had been managed without the use of inorganic fertilizers and only limited use of pesticides (that is spot spraying mainly around field edges and in ditches) since the farm was first ploughed in 1909. The organic farm relied upon green manure crops, crop rotations, and native soil fertility for plant nutrients. The organic farm used a crop rotation of winter wheat (*T. aestivum*), spring pea (*Pisum sativum*) and Austrian winter pea (*P. sativum* spp. *arvense* L. Poir). The Austrian winter peas, which were used as a green manure crop, were replaced by summer fallow in dry years (about every sixth year).

The adjoining 525 ha conventional farm was first cultivated in 1908 but did not begin receiving recommended rates of fertilizers and pesticides until 1948 and the early 1950s, respec-

Table 1 Estimates of recent winter wheat yields

Year	Organic farm (tons ha ⁻¹)	Conventional farm (tons ha ⁻¹)	Second conventional* farm (tons ha ⁻¹)
1982	4.79	4.85	4.05
1983	4.25	4.92	3.91
1984	4.25	5.26	4.05
1985	4.38	4.72	3.98
1986	4.85	4.72	3.98
Average	4.50	4.90	3.99

Estimates for all three farms were derived from the farm managers and the USDA Agricultural Stabilization and Conservation Service in Spokane, Washington. Both conventional farms were adjacent to the organic farm.

* This second conventional farm of 328 hectares used an annual cropping rotation (no fallow) of winter wheat, Steptoe barley and spring pea.

Table 2 Mean values of soil properties

Soil property	Organic farm	Conventional farm
Surface soil colour	10YR 4/2	10YR 5/2, 5/3
Polysaccharide content (g kg ⁻¹ soil)	1.13*	1.00
Moisture content (%)	15.49†	8.98
Modulus of rupture (MPa)	1.61 × 10 ⁻²	1.98 × 10 ⁻² *
Surface texture	Silt loam	Silt loam
Subsoil (Bt) texture	Silty clay loam	Silty clay loam
Bulk density (mg m ⁻³)	0.98	0.95
Surface (A1) horizon thickness (cm)	39.80*	36.68
Depth to argillic horizon (cm)	55.60†	39.80

The initial study area consisted of a pair of transects, with ten sample points in each transect. Each transect was parallel to and 4.5 m from the boundary line (between the farms) and 55 m long. Soil samples were collected in the summer of 1985 from the surface 0–10 cm for all 20 samples and analysed for bulk density and soil water content. At the same 20 sample points, deeper soil cores (7 cm diameter) to at least 100 cm in depth were analysed in the field for soil texture, colour, thickness of A1 horizon and depth to the Bt horizon. From the texture and colour changes evident in the soil core profiles, thicknesses of the surface horizons to the underlying argillic (Bt) horizons were determined. A *t*-test was used to compare the bulk densities, moisture contents, thickness of the A1 horizons, and depths to the Bt horizons between the two farms. The study area also consisted of four plots (6.1 × 9.1 m), two on each side of, and 6.1 m away from the boundary line between the farms. Twenty surface soil samples (0–10 cm depth) were collected from a grid pattern superimposed on each of the four plots for a total of 80 samples. These samples were analysed for modulus of rupture using the Reeve method²⁶ and polysaccharide content according to the anthrone method proposed by Brink *et al.*²⁷ and modified by Metting and Rayburn²⁸. Statistical comparisons of the results were made using ANOVA.

* <0.05.

† <0.01.

tively (A. Clausen, personal communication). Winter wheat received N, P, and S applications at 96, 34, and 16 kg ha⁻¹, respectively; spring peas received no fertilizer. The conventional farm generally used a crop rotation of winter wheat (*T. aestivum*) and spring pea (*P. sativum*). Summer fallow was used when necessary for water conservation in dry years.

Compared to the conventional farm management system, the organic system had similar tillage operations when growing peas but fewer tillage operations when growing wheat (no fertilizer operation) and when green manuring. The two management systems had similar crop varieties but differed in fertilizer use, crop rotations, and managers. Average winter wheat yields for the past five years were 8% lower on the organic farm than on the conventional farm, but almost 13% higher than on a second

conventional farm (with similar soils) adjacent to the organic farm (Table 1).

The soil properties investigated are shown in Table 2. The surface layer of the organically-farmed soil was darker (10YR 4/2 or dark greyish brown) than the surface layer of the conventionally-farmed soil (10YR 5/2 or greyish brown, and 10YR 5/3 or brown), indicating significantly higher organic matter levels in the organically-farmed soil. An earlier study⁸ with the two farms showed that the organically-farmed soil had significantly higher levels of organic carbon. These results support the conclusion that organic farmers can, and generally do, achieve higher organic matter levels in their soils than do conventional farmers^{9,10}.

Organic matter has a profound impact on soil quality; it encourages granulation, increases water storage, nutrient supply, and soil organism activity, and improves soil fertility and productivity^{11,12}. An earlier study⁸ with these two farms showed that the organically-farmed Naff soil had significantly higher levels of urease, phosphatase, and dehydrogenase (soil enzymes) and significantly higher microbial biomass. We found that the organically-farmed soil also had significantly higher polysaccharide content than the conventionally-farmed soil (Table 2). Polysaccharides, some of which are produced by soil microorganisms, serve as active binding agents in soil aggregate formation, and are involved in aggregate stability^{13,14}.

Moisture contents were significantly higher in the organically-farmed soil than in the conventionally-farmed soil (Table 2), which may be attributed to the higher organic matter levels of the organically-farmed soil. As moisture-release curves from an earlier study¹⁵ were similar for both soils, it may be inferred that the water potential of the organically-farmed soil was higher than that of the conventionally-farmed soil at the time of sampling. The organically-farmed soil had a significantly lower modulus of rupture (an index related to the hardness of surface crusting), indicating that seedling emergence could be enhanced in the organically-farmed soil. Surface horizons of both farms had silt loam textures, whereas the argillic horizons had silty clay loam textures (Table 2). Bulk densities of the organically and conventionally-farmed soils were not significantly different.

The surface horizon of the organically-farmed soil was significantly thicker (by 3 cm) than the surface horizon of the conventionally-farmed soil. Topsoil thickness, represented by the depth of the soil layers (A1 and A2 horizons) to the subsoil argillic horizon, was almost 16 cm greater in the organically-farmed than conventionally-farmed soil. These differences, especially the large difference in topsoil depth, are attributed to significantly greater soil losses due to erosion on the conventionally-farmed soil between 1948 and 1985.

Erosion not only reduces the surface horizon thickness but also, more significantly, brings the subsoil layer(s) (in this case the argillic layer) nearer to the surface (Fig. 1). Despite the higher erosion rate of the conventionally-farmed Naff soil, its surface (A1) horizon thickness was only 3 cm thinner than that of the organically-managed soil. This was due to its continual mixing with the A2 horizon by ploughing, and the addition of fresh organic matter from crop residues. But the A2 layer was much thinner, so the amount of productive topsoil was dramatically less (by 16 cm) on the conventionally-farmed soil. This loss of topsoil was due to water and tillage erosion.

This conclusion is supported by the results of water erosion research conducted on the same study area¹⁵, examining both the organic and conventional farms using the Alutrin method to measure the cross-sections of rills, in a year when both farms were in winter wheat. Water erosion was found to be 8.3 ton ha⁻¹ on the organic field and 32.4 ton ha⁻¹ on the conventional field, almost a fourfold difference.

The rate of water erosion on the conventional field is very close to the average annual rate of water erosion of 31.5 ton ha⁻¹ in the Palouse area, one of the more erosive areas in the United States¹⁶ and is almost three times the maximum soil-loss toler-

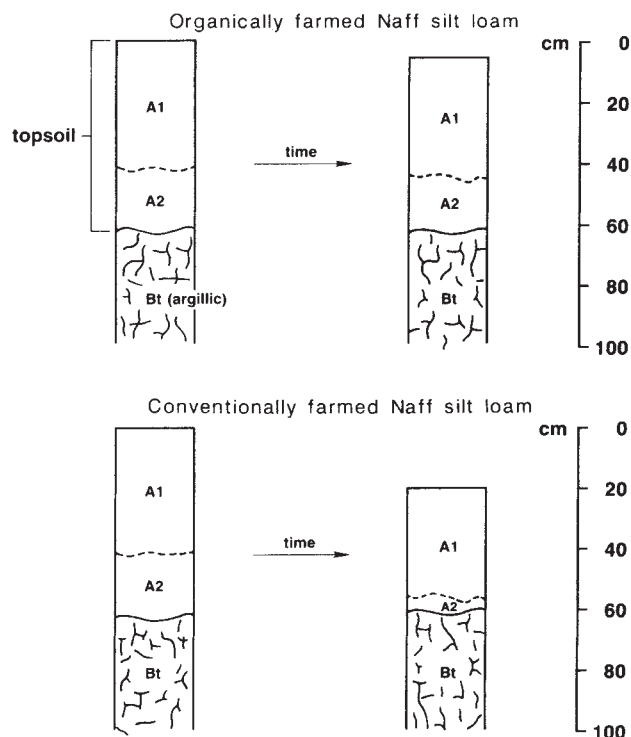


Fig. 1 Organically and conventionally farmed soil losses due to water erosion between 1948 and 1985. The organically farmed Naff silt loam has lost about 5 cm of topsoil, while the conventionally farmed Naff silt loam has lost about 21 cm of topsoil leaving a 16 cm difference in topsoil thickness between the two soils in 1985. The A2 horizon for Naff soils is characteristically only slightly lower in organic matter than the A1 horizon. So its mixing with the thinning A1 by ploughing, plus the addition of organic matter from crop residues, helps maintain a fairly normal A1 horizon until the A2 horizon is completely depleted. Topsoil losses were extrapolated from both field measurements of water erosion¹⁵ and topsoil thickness (Table 2). The effects of tillage erosion on topsoil thickness were not included.

ance value of 11.2 ton ha⁻¹ yr⁻¹ for Naff soils, whereas the rate of water erosion on the organic farm is <75% of the maximum tolerance value¹⁷. Soil loss tolerance, called the 'T value', is the maximum rate of soil erosion that can occur without reducing long-term crop productivity or environmental quality of a specific soil¹⁸. Our data indicate that the long-term productivity of the organically-farmed Naff soil is being maintained, whereas that of the conventionally-farmed Naff soil is being reduced because of high rates of soil erosion.

Loss of topsoil by erosion has been shown to reduce organic matter, fine clays, available water-holding capacity, plant rooting depth, soil productivity, and crop yields^{19,20}. At current rates of water and tillage erosion for typical Naff and similar soils of varying slope positions under conventional farming systems, it has been projected that all the topsoil will be lost in 50 yr, exposing the denser, less fertile subsoil argillic horizons²¹. Also, crop yields will be reduced at zero topsoil to 2.4 ton ha⁻¹ and 1.8 ton ha⁻¹ for Naff soil on Class III and IV sites respectively²¹. Our study area is on a Class III Naff soil.

These projected declines in yield ignore any concurrent yield increases due to improved technology over this 50-yr period. But uncontrolled erosion can substantially decrease the benefits from improved plant varieties and cultural practices, which have the greatest potential for increasing winter wheat yields on deep, relatively uneroded topsoils²². At some point, the increasing yield reduction from erosion may exceed the diminishing yield increase due to technical progress.

The difference in erosion rates between the organic and conventional farms was most probably due to their different crop rotation systems. Only the organic farm included a green manure legume crop in the third year of rotation, and it had fewer tillage operations. Comparisons of erosion rates for monoculture plots or non-legume-based crop rotation plots (typical of many conventional farming systems) versus legume-based crop rotation plots (typical of organic farming systems) have indicated a significant reduction in soil erosion due to legume-based crop rotations²³⁻²⁵. The benefits from green-manuring with a legume crop and reducing tillage operations could be included in the conventional farming system as well as a reduced level of commercial fertilizers. If conventional farming methods are not modified, the loss of valuable Naff topsoil will continue.

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A behavioural method for accelerating re-entrainment of rhythms to new light-dark cycles

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The idea of ameliorating jetlag with drugs has received considerable attention. Melatonin has been found to reduce feelings of jetlag in people after transatlantic flights¹. In hamsters, injections of triazolam, a benzodiazepine, increase the rate of adjustment of activity rhythms to an 8 h advance of the light-dark (LD) cycle². But melatonin can make people drowsy and triazolam often induces hamsters to run in their wheels^{2,3}. Therefore, it is not clear whether these chemicals exert their chronotypic effects by acting directly on circadian pacemakers or because they first alter behavioural states. Non-photoc behavioural events (for instance, social interactions) are capable of entraining rhythms and causing phase shifts⁴. Thus, it is possible that behavioural events alone could alter the rate of adjustment to new LD cycles. To investigate this possibility, we studied the rate of re-entrainment of hamsters in a testing paradigm similar to that used with triazolam². We found that the rate of adjustment could be more than doubled simply by making the animals active on a single occasion in the middle of their normal rest period, immediately after the shift in the LD cycle.

Male hamsters (*Mesocricetus auratus*, LVG Charles River, Montreal, aged 95 days on the day of the first test) were kept in cages containing activity wheels⁵. They were housed in a room with a cycle of 14 h light and 10 h dark. Illumination in the cages during the light period was ~100 lux, as measured with a Gossen Lunasix light meter. During the dark period there was dim red illumination of about one lux. To accustom the animals to these conditions, they were kept in cages with activity wheels for ten days, and in the LD cycle for 30 days before test 1. After stable entrainment was obtained, all animals were subjected to an 8 h phase advance in the LD cycle, starting with

an advance in the onset of darkness. Half the animals ($n = 10$) were left undisturbed. The other half ($n = 10$) were removed from their cages 1 h after the new onset of darkness and confined to running wheels. These wheels were not the same ones as used in the home cages, were clean, and were located in a different part of the room. After 3 h, the animals were returned to their home cages. Note that this was not a periodic daily event but occurred only on the day of the shift in the LD cycle.

Twenty days later, when all animals had adjusted their activity to the new LD cycle, the procedure was repeated, using the animals that had been undisturbed in the first test as the test animals; those animals that were confined to running wheels in test one were left undisturbed. The only other difference was that six days before the second test, the red lamps were removed, making the dark period totally dark. Manipulations were carried out with the aid of an infrared nightscope.

All the test animals ran vigorously when confined to the new wheels; they adjusted more quickly to the shift in the LD cycle than the controls (Fig. 1). Almost all of the control animals remained asleep or quiescent during the 3-h test period. To specify when resynchronization had occurred, we used the number of days required for an animal to begin its wheel-running within 30 min. of the onset of darkness on the new LD cycle, as in ref. 2. Undisturbed animals took about 8.5 days for re-entrainment while those that had the additional 3 h wheel running took only 1.5 days (Table 1). But the control animals adjusted faster in test 1, when the dim red light was present throughout. Age, experience and changing susceptibility to disturbance from neighbours that had already phase-shifted are other factors that might be involved. Whatever the explanation, in both tests, the experimental animals adjusted far more quickly

Table 1 Days taken to resynchronize (mean $\pm$ s.e.m.)

	<i>n</i>	Undisturbed	3 h extra activity	<i>P</i> *
Test 1	10	5.4 ($\pm$ 0.67)	1.6 ($\pm$ 0.31)	<0.01
Test 2	10	11.6 ($\pm$ 0.73)	1.5 ($\pm$ 0.17)	<0.01
Total	20	8.5 ($\pm$ 0.86)	1.6 ($\pm$ 0.17)	

* Two-tailed *t*-test.

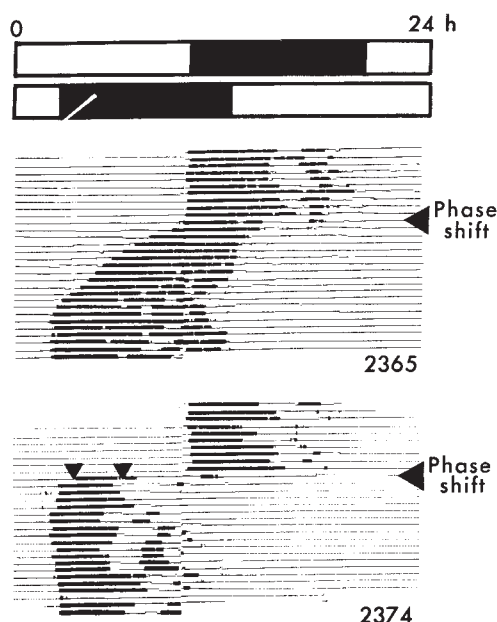


Fig. 1 Activity records for a typical animal from the control group (top) and from the group with 3 h of extra activity (bottom) in test 2. Each line shows 24 h, with each day mounted below the previous day. Black marks indicate wheel-running. The two downward-pointing arrows indicate when the experimental animals were confined to the extra activity wheel, which was 17.5 cm in diameter with no exits. This treatment began 1 h after the advanced onset of darkness and lasted 3 h; other than this single 3-h period all wheel-running shown is in the animal's home cage. Open and solid horizontal bars depict the light-dark cycles prevailing before and after the 8-h phase shift.

than the controls. In test 2 all the additional activity occurred during total darkness, thus eliminating any photic input during the time of the manipulation.

Although it is clear that being in the running wheels for three hours greatly increased the rate of phase-shifting, it is difficult to tell what aspect of this experience was most important. As well as exercising during this time, the hamsters were in a novel environment and were awake at a time when they would normally have been sleeping. It is difficult to isolate the critical variables because waking an animal is also likely to alarm it and to make it more active. Possibly wheel-running is effective because it both keeps the animal awake and involves intense motor activity. It also remains an open question whether behavioural events and benzodiazepines act independently on the same mechanism or whether benzodiazepines are effective in phase-shifting hamsters because they result in activity. In either case, the results demonstrate that behavioural events in themselves can greatly accelerate the rate of resynchronization to new LD cycles.

After people have crossed time zones, outdoor activity helps them to adjust faster⁶. It is not possible to tell from those studies whether it was activity or exposure to more light (or both) that was important. If activity can induce phase shifts in human circadian rhythms, then it may be possible to design exercise schedules for situations in which human circadian rhythms are desynchronized from local time cues, such as jetlag. This suggestion is analogous to that arising from studies of the effects of chronotropic drugs on animals⁷. For the fit and pharmacologically conservative, jogging might be preferable to drugs. However, activity would have to be scheduled at appropriate times during the circadian cycle, otherwise it could retard adjustment⁴. It might also be worth considering the possible implications of these findings for the scheduling of sporting events, shift work involving strenuous activity, and the treatment of

abnormal phase relationships in some cases of affective disorder where drug therapy is contraindicated.

The present results are also of some theoretical interest. Traditionally, a distinction is made between the overt measurable rhythm (the hands of the clock) and the pacemaker (the clock mechanism itself); the overt rhythm is considered as subordinate to the pacemaker. Perhaps in certain circumstances the clock may be susceptible to feedback from overt activity.

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Hormonal regulation of K⁺-channel messenger RNA in rat myometrium during oestrus cycle and in pregnancy

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The dramatic enhancement of smooth-muscle excitability in the uterus which occurs at oestrus and at term in pregnant rats is closely related to increased blood oestrogen concentrations¹⁻⁴. How oestrogen alters the electrical properties of myometrial cells is unclear, although electrical coupling between cells has been shown to increase⁵⁻⁹. Many examples are known of changes in cellular excitability involving modification of existing ion channels by second-messenger pathways¹⁰. Steroid hormones, in contrast, are generally thought to influence cellular processes mainly through effects on gene expression, inducing the synthesis of new proteins¹¹⁻¹². Previous work, using an oocyte translation system¹³⁻¹⁵, has shown that a very slowly activating, voltage-dependent K⁺ current can be expressed from the poly(A)⁺ RNA of oestrogen-treated rat uteri¹⁶. We report here that the messenger RNA species producing this channel is rapidly and reversibly induced in the presence of oestrogen, as shown by the appearance and disappearance of this mRNA during the oestrous cycle, its emergence at the end of pregnancy, and its presence or absence following hormonal treatments. These results suggest that oestrogen controls the expression of a voltage-dependent ion channel in uterine smooth muscle cells.

The right-hand traces in Fig. 1a illustrate the very slowly activating voltage-dependent K⁺ current that is expressed in *Xenopus* (toad) oocytes injected with poly(A)⁺ RNA from the uteri of ovariectomized rats treated with oestrogen. The properties of this current correspond closely¹⁶ to those of slowly activating K⁺ channels in visceral smooth-muscle cells¹⁷. During a depolarizing voltage-clamp pulse of several seconds, this potassium conductance becomes activated, producing an outward current; following the pulse, it relaxes with a decay time of many seconds, resulting in a very slowly decaying outward tail current. In the experiments described here, the amplitude of the slow tail current following a standard depolarizing step, rather than the current during the step itself, was used for comparisons, giving a measure of the expressed current unlikely to be affected by endogenous currents¹⁶. The K⁺ current is

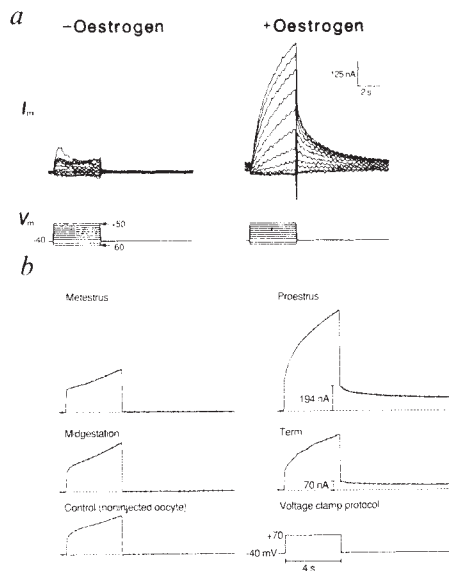


Fig. 1 Expression in *Xenopus* oocytes of uterine RNA from oestrogen-treated and non-oestrogen-treated, cycling, and pregnant rats. *a*, Voltage-clamp currents recorded from oocytes (from a single donor) injected with poly(A)⁺ RNA from oestrogen-treated (+Oestrogen) and non-oestrogen-treated (-Oestrogen) rat uteri. *b*, Voltage-clamp currents recorded from oocytes (from same donor as in Fig. 2*a*) injected with RNA from cycling (metoestrus and pro-oestrus) and pregnant (midgestation and term) uteri and a noninjected control oocyte.

Methods. In *a*, ovariectomized rats were either implanted with silastic capsules containing 100% oestradiol, or not given any treatment, and then killed after three days¹⁶. In *b*, vaginal smears were taken and only those rats showing at least two consecutive four-day oestrus cycles were taken to be 'cycling'. Timed pregnant rats (Charles River CD[SD]Br strain) were killed either on day 15 ('midgestation') or on day 22, before delivery occurred ('term'). The uterine horns were immediately frozen in liquid nitrogen, and the RNA made by a modification of the guanidine hydrochloride method²³. The yield of poly(A)⁺ RNA was similar (3–6% of total RNA) for all batches of uterine RNA in our experiments, except those from the midgestational uterus (two samples, 10 and 13% of total RNA). For oocyte injection, *Xenopus* oocytes were prepared as previously described¹⁶ and injected with 200 ng poly(A)⁺ RNA. The oocytes were incubated for six days at 19 °C in Barth's medium. Conventional two-microelectrode voltage-clamp techniques were used and all recordings were made at room temperature (23–25 °C). In *a*, the bathing solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES, pH 7.6. The oocytes were depolarized for 4 s to a range of test voltages from a holding potential of -40 mV. Currents have been leak-subtracted in *a* only. In *b*, recordings were made under Na⁺- and Ca²⁺-free conditions to minimize the possible contribution of inward components of current; consequently, the control currents differed somewhat from those recorded in *a*. The bathing solution contained 100 mM *N*-methyl-D-glucamine, 2 mM KCl, 1 mM MgCl₂, 0.1 mM EGTA, 5 mM HEPES, pH 7.6. The oocytes were depolarized for 4 s from -40 mV to +70 mV and then returned to -40 mV. The peak amplitude of this tail current was measured from a baseline (broken line) defined by the current shown at -40 mV before the depolarizing step was applied.

expressed to a similar degree in oocytes whether or not the endometrium is removed by dissection before the RNA is made, implying that the mRNA species responsible for the expression of this channel originates in cells of the myometrium. Oocytes injected with RNA from the uteri of ovariectomized rats not given oestrogen (Fig. 1*a*, left) show only currents similar to those recorded from uninjected control oocytes.

The mRNA species responsible for the expression of the slowly activating current seems to be induced during physiological increases in excitability (Fig. 1*b*). The current can be expressed from uterine RNA extracted at the times during pregnancy and the oestrus cycle when oestrogen concentrations and the excitability of the smooth muscle are high, but not at other times. Concentrations of oestrogen are highest during pro-oestrus, the day preceding oestrus, when frequent uterine movements occur, but are low at metoestrus¹⁸. During pregnancy, oestrogen is the dominant hormonal influence only at term, as the uterus shifts from a quiescent state to a highly

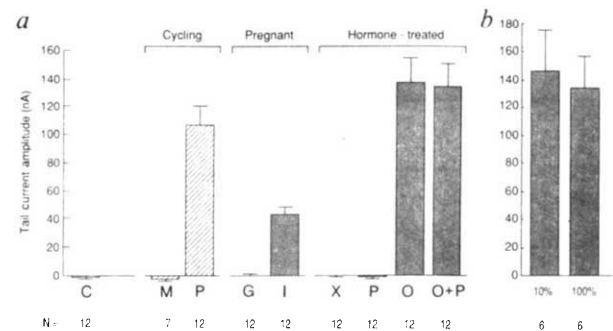


Fig. 2 Slow K⁺ current expression in oocytes injected with RNA from uteri of pregnant, cycling, and hormone-treated rats. Values in parentheses show the number of animals tested. *a*, Mean tail current amplitudes ($\pm$ s.e.m.) are shown for groups of oocytes (all from the same donor) injected with 200 ng poly(A)⁺ uterine RNA from cycling (M, metoestrus; P, pro-oestrus), pregnant (G, midgestation; T, term) or hormone-treated (X, ovariectomized; P, progesterone-treated; O, treated with oestrogen; O+P, treated with oestrogen and progesterone) rats and incubated for six days. C, control, uninjected oocytes. The mean resting potentials and input resistances for these groups are shown in Table 1. *b*, Slow outward tail currents in oocytes (from another toad) injected with uterine RNA from animals treated with oestrogen to produce 'pro-oestrus-like' (10% oestradiol capsules) or 'term-like' (100% oestradiol capsules) elevations of blood concentrations. The mean resting potentials and input resistances ($\pm$ s.e.m.) were -56.7 ± 1.35 mV and 0.55 ± 0.06 M for the 10% group and -55.2 ± 0.59 mV and 0.58 ± 0.10 M for the 100% group.

Methods. In *a*, rats given hormone treatment were cycled as described for Fig. 1, ovariectomized on the day of pro-oestrus and implanted with capsules containing 100% oestradiol¹⁶, or crystalline progesterone (internal diameter 3.35 mm; external diameter 4.70 mm; length 1.5 cm). In *b*, rats were ovariectomized and implanted with silastic capsules containing either 100% oestradiol¹⁶, or a mixture of 10% oestradiol and 90% cholesterol²⁰. The oocytes were incubated for three days after RNA injection before electrical recordings were made.

active one¹⁹. The slowly activating current was expressed in oocytes injected with RNA from term-pregnant and pro-oestrus uteri (Figs 1*b* right and 2*a*), but was not detected in oocytes injected with RNA from midgestational or metoestrus uteri, which displayed only currents similar to those seen in uninjected control oocytes from the same donor (Figs 1*b* left and 2*a*).

Because pro-oestrus and metoestrus occur only two days apart in the four-day oestrus cycle, it seems that the concentration of the mRNA producing the current can be rapidly increased and decreased in the myometrial cells in response to changes in oestrogen levels. Uterine RNA from animals ovariectomized at pro-oestrus and given no hormonal treatment yielded no expression (Fig. 2*a*), indicating that the mRNA species, initially present in detectable amounts in the pro-oestrus uterus, is lost from the myometrial cells within a few days of oestrogen withdrawal. Treatment with the gonadal steroid hormone progesterone, concentrations of which also vary with the ovarian cycle and during pregnancy, but which is generally associated with reduced, rather than enhanced, electrical excitability, did not prevent loss of expression or itself induce expression. The slow current was detected only using RNA from animals in which a high concentration of blood oestrogen had been maintained. The RNA from animals treated with oestrogen alone or a combination of oestrogen and progesterone gave expression of the current comparable to that for the pro-oestrus RNA injected into oocytes from the same toad (Fig. 2*a*). A similar protocol involving a combination of oestrogen and progesterone results in the expression of myometrial electrical properties like those of the term pregnant uterus³.

The peak blood oestrogen concentrations produced by oestrogen treatments such as those of the experiments in Figs 1*a*

Table 1 Membrane potential and input resistances of oocytes used to test RNA

Source of RNA injected	V_m (mV)	R_{in} (M Ω)
None (control noninjected)	-45.5 ± 1.5	1.1 ± 0.06
Ovariectomized, no hormone treatment	-44.3 ± 0.6	1.2 ± 0.06
Metoestrus	$-49.9 \pm 1.8^*$	1.2 ± 0.04
Midgestation	$-54.5 \pm 1.5^{***}$	1.2 ± 0.06
Ovariectomized, progesterone-treated	$-49.9 \pm 1.1^{***}$	1.1 ± 0.09
Pro-oestrus	$-59.3 \pm 0.8^{***}$	$0.6 \pm 0.03^{***}$
Term	$-60.8 \pm 1.0^{***}$	$1.0 \pm 0.06^*$
Ovariectomized, oestrogen-treated	$-58.0 \pm 1.1^{***}$	$0.8 \pm 0.06^{**}$
Ovariectomized, treated with oestrogen and progesterone	$-61.7 \pm 1.3^{***}$	$0.4 \pm 0.06^{***}$

Membrane potentials (V_m) and input resistances (R_{in}) were measured in the Na^+ - and Ca^{2+} -free solution described in Fig. 1b legend. Input resistance was measured using a 10-mV hyperpolarizing pulse from -55 mV to -65 mV. Values are means $\pm$ s.e.m. and the number of determinations in each group is as in Fig. 2. One-way analysis of variance indicated significant differences among the group means for both resting potential ($F = 34.98$, $P < 10^{-6}$) and input resistance ($F = 24.96$, $P < 10^{-6}$). Student's t -tests were used to determine which group means were significantly different from those of the group not treated with hormone: * $P < 0.05$; ** $P < 10^{-3}$; *** $P < 10^{-4}$.

and 2a are 100–150 pg ml $^{-1}$, similar to those in late pregnancy¹⁹ but higher than those at pro-oestrus²⁰. We therefore compared the ability of treatments producing moderate ('pro-oestrus-like') and high ('term-like') increases in blood oestrogen concentration to induce the mRNA responsible for the K^+ channel. Figure 2b shows that uterine RNA from ovariectomized animals treated to produce 'pro-oestrus-like'²⁰ amounts of oestrogen in the blood (30–50 pg ml $^{-1}$) also gave expression of the current, indicating that oestrogen concentrations of the magnitude of those that occur at pro-oestrus can account for the expression of the slowly activating current seen using pro-oestrus RNA. That the amount of current expressed in oocytes injected with RNA of term pregnant uteri was less than for oestrogen-treated uteri may be due to the fact that at term much of the maternal blood oestrogen is not free but is bound by α -fetoprotein²¹.

Oocytes which expressed the current also had more negative resting potentials than control oocytes, as might be expected from an increase in potassium conductance (Table 1). Oocytes injected with RNA from progesterone-dominated uteri (progesterone, midgestation, metoestrus), which showed no detectable expression of the slowly activating current, also had more negative resting potentials than oocytes injected with RNA from ovariectomized rats given no hormone treatment (Table 1). In contrast to oocytes expressing the current, the membrane conductances in the oocytes from the progesterone groups were not increased, suggesting that the more negative resting potentials are not caused by new ion channels in the plasma membrane. The more negative membrane potentials in oocytes from the progesterone groups were not blocked by 100 μ M ouabain during a 10-min exposure ($n = 3$).

These experiments show that in the myometrium, which undergoes profound alterations in electrical excitability, oestrogen rapidly and reversibly increases the concentration of an mRNA species that gives rise to K^+ channels in *Xenopus* oocytes. The most likely explanation for these observations is that oestrogen controls the concentration of the mRNA species encoding this uterine K^+ channel, but it is also possible that oestrogen regulates an mRNA species encoding a modulatory protein required for the expression of the K^+ current. Increased expression of this current in uterine smooth muscle cells may account for the marked hyperpolarization of membrane potential observed after oestrogen treatment^{3,22}. Changes in the expression of voltage-dependent ion channels may be funda-

mental mechanisms for the regulation of excitability.

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Adenohypophyseal degradation of thyrotropin releasing hormone regulated by thyroid hormones

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Thyrotropin Releasing Hormone (TRH; pyroGlu-His-Pro-NH $_2$) is important in the regulation of adenohypophyseal hormone secretion^{1,2} and also serves neurotropic functions in extra-hypothalamic brain areas^{3,4}, indicating that it is involved in neurotransmission and other forms of cellular communication. This hypothesis is strengthened by the observation that TRH is hydrolysed at the pyroGlu-His bond by a particulate^{4,5} enzyme located in the synaptosomal⁴ and adenohypophyseal plasma membrane⁶. Furthermore, this enzyme has been identified as a heterogeneously distributed ectoenzyme⁷ which has a high degree of substrate specificity⁸ like the TRH-degrading serum enzyme studied previously⁹. In the rat, the activity of the TRH-degrading serum enzyme has been shown to be influenced by the thyroid status of the animals^{10,11}; here I report that the activity of the membrane-bound TRH-degrading enzyme of the anterior pituitary is stringently controlled by thyroid hormones, but that the activity of the brain enzyme is not.

Figure 1 shows the time course of degradation of 3H -TRH by washed membrane homogenates. When membrane homogenates were prepared from untreated animals, the tripeptideamide was most rapidly degraded with brain homogenates (Fig. 1), hypothalamic tissue preparations had less activity and the adenohypophyseal membranes degraded TRH only slowly (18 times less rapidly than brain membranes). When the animals were treated with the mild goitrogenic agent propylthiouracil (PTU), a rapid loss of activity of the membrane-bound adenohypophyseal enzyme was observed (Fig. 2): after 4 days of treatment the enzyme activity was reduced by 50%, and after 16

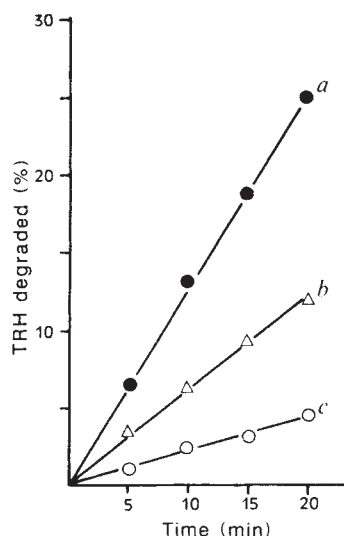


Fig. 1 Time course of degradation of TRH (refs 14 and 15) by washed membrane homogenates of *a*, brain (10 μ l, 102 μ g protein), *b*, hypothalamus (10 μ l, 98 μ g protein), *c*, anterior pituitary (30 μ l, 230 μ g protein). Incubation (final volume: 50 μ l) with 3 H-TRH (0.1 μ Ci) at 30 $^{\circ}$ C was for time periods indicated.

Methods. Male Sprague-Dawley rats (300–350 g body weight; 6 animals per group) were used in all experiments. After appropriate treatment, the animals were killed by decapitation. The tissues were quickly dissected and homogenized in 10 vols of buffer (25 mM sodium phosphate, pH 7.4 containing 0.04% NaN_3). After centrifugation at 100,000g for 1 h, the pellets were rehomogenized and centrifuged again to ensure that the membrane preparation was not contaminated by the TRH-degrading serum enzyme. The pellets were rehomogenized in 4 vols buffer containing 1 mM diisopropyl fluorophosphate to inhibit the membrane-bound dipeptidyl aminopeptidase IV, and thus avoid further catabolism of His-Pro-NH₂, the primary cleavage product of TRH. TRH was radiolabelled in the proline or pyroGlu moiety and used as substrate, its degradation being determined as previously described^{14,15}. For comparison, the tissue extracts were also tested to determine the activities of the cytosolic TRH-degrading enzymes, pyroglutamate aminopeptidase and proline endopeptidase using the substrates pyroglutamyl- β -naphthylamide¹⁶ and N-Cbz-Gly-Pro- β -naphthylamide¹⁷ respectively. The liberation of β -naphthylamine was determined fluorometrically^{16,17} and protein concentration was determined by a modification of the Lowry method¹⁸ using bovine serum albumin as standard.

days only 15% of the control activity remained. For comparison, the activity of the TRH-degrading serum enzyme was only reduced by 40% after 16 days of PTU treatment, indicating that the adenohipophyseal enzyme is affected directly. In contrast, the activities of the membrane-bound enzymes from brain and hypothalamic tissue were unaffected by PTU treatment, as were the activities of the cytosolic TRH-degrading enzymes from adenohipophyseal tissue and brain. PTU itself had no effect on the activities of any of the enzymes tested *in vitro*.

When the animals received a single subcutaneous injection of only 10 μ g triiodothyronine (T_3) per 100 g body weight, the activity of the membrane-bound adenohipophyseal enzyme rapidly increased (Fig. 3). The initial lag period of 4 h indicates that the onset of hormone expression is probably preceded by the induction of protein synthesis, as has been observed in other cases for the induction of cellular activities by thyroid hormones¹². After this short lag phase¹² the activity of the membrane-bound adenohipophyseal enzyme increased rapidly by about 30% per hour and reached a maximum (550% of control) after 24 h. Thereafter, its activity rapidly declined, to reach control values after about 90 h. In parallel with its biological activity, only a twofold stimulation of this enzyme activity was observed 24 h after injection of 10 μ g thyroxine (T_4) (data not shown). These results indicate that thyroid hormones exert a

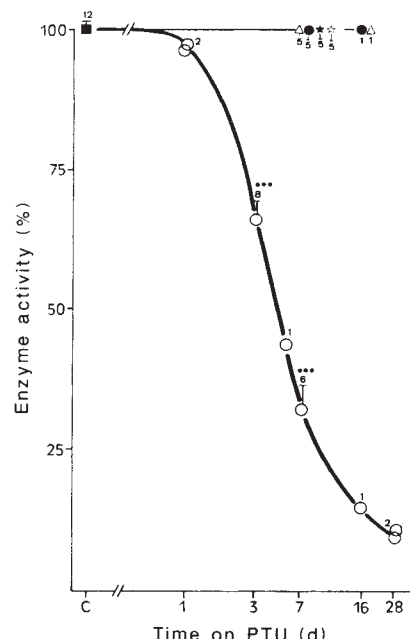


Fig. 2 Effect of propylthiouracil (PTU) on the activities of TRH-degrading enzymes. The animals were treated with PTU (200 mg l^{-1} drinking water) for various times. Washed membrane homogenates and tissue extracts were prepared as described in Fig. 1 legend. For the membrane-bound enzymes ($\circ$, $\bullet$, Δ) the initial rate of TRH degradation as a measure of enzyme activity was determined by a 4-point kinetic, as described in Fig. 1. $\circ$, Anterior pituitary; $\bullet$, brain; Δ , hypothalamus. The activities of the soluble TRH degrading enzymes ($\star$, $\star$) of the adenohipophysis were determined by fluorometric assays^{16,17}. $\star$, Pyroglutamate aminopeptidase; $\star$, proline endopeptidase. Control represents 100% enzyme activity. The numbers indicate the number of experiments performed, and the dots above the number, the statistical values (Student's *t* test; $\bullet\bullet$, $P \leq 0.01$; $\bullet\bullet\bullet$, $P \leq 0.001$).

direct effect on the activity of the membrane-bound adenohipophyseal enzyme, unlike the serum enzyme whose activity increases significantly only when thyroid hormones are injected repeatedly for long periods of time¹¹.

In vitro, T_3 itself did not directly affect the activity of any of the enzymes tested. As expected from the results with PTU, the activities of both the adenohipophysis and brain soluble TRH-degrading enzymes and of the membrane-bound TRH-degrading enzymes from brain and hypothalamic tissue were found to be unaffected by treatment of the animals with thyroid hormones. The activities of these enzymes were also unchanged when the animals received either multiple injections of 10 μ g T_3 per 100 g body weight for 96 h (data not shown) or higher doses of T_3 (Fig. 4). In contrast, the effect of T_3 on the activity of the membrane-bound enzyme from the anterior pituitary is strongly dose-related: even after a single injection of only 0.5 μ g T_3 per 100 g body weight, a significant increase in activity was seen after 24 h.

In agreement with a recent report¹³, these results demonstrate that the activity of the membrane-bound TRH-degrading brain enzyme is not controlled by thyroid hormones. Nevertheless, current data indicate that it serves specialized functions and could thus be important for the transmission of TRH signals; analogy to acetylcholine esterase is evident, but further studies will be necessary to investigate whether this enzyme represents the peptidergic equivalent to acetylcholine esterase.

The findings that the membrane-bound adenohipophyseal TRH-degrading enzyme is controlled by thyroid hormones suggest that the enzyme itself could serve as a regulatory control element to influence the extent and duration of the endocrine activities of TRH at adenohipophyseal target sites, and hence the secretion of adenohipophyseal hormones. The rapid onset

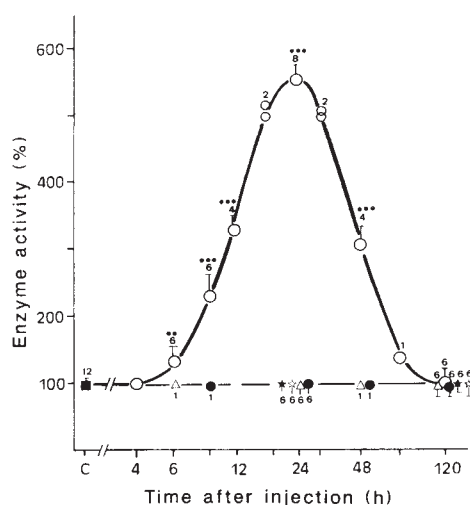


Fig. 3 Effect of triiodothyronine (T_3) on the activities of TRH-degrading enzymes. Control represents 100% enzyme activity. **Methods.** The animals were injected subcutaneously with $10 \mu\text{g}$ of T_3 per 100 g body weight. After various times, groups of 6 animals were killed. Washed membrane homogenates and tissue extracts were prepared as described in Fig. 1 legend. Enzyme activities were determined as described in legend to Fig. 2. For explanation of the symbols, see Fig. 2.

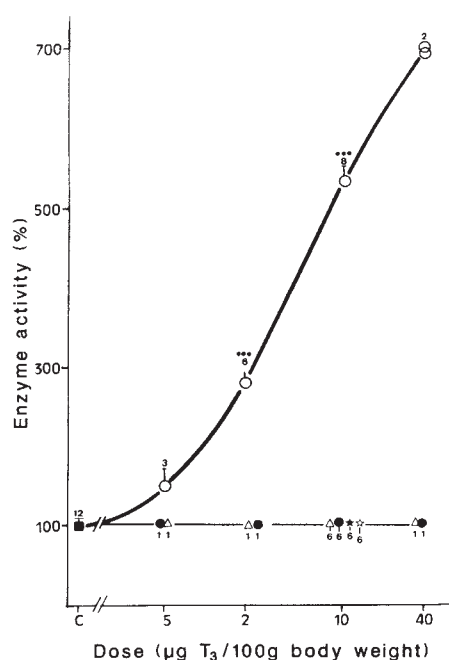


Fig. 4 Dose-relationship of T_3 on the activities of TRH-degrading enzymes. Control represents 100% enzyme activity. The animals were injected subcutaneously with the amount of T_3 as indicated and killed 24 h later. The membrane homogenates and tissue extracts were prepared and the enzyme activities were determined as in Fig. 2. The same symbols were used as for Fig. 2.

in the induction of this enzyme and the rapid decline in its activity within physiologically significant time periods indicate a high turnover of the enzyme, and thus support the concept of specialized functioning of the membrane-bound TRH-degrading enzyme of the adenohypophysis; but further studies are needed to define the functions of the membrane-bound TRH-degrading enzymes at their specific sites.

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Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm

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In most species the length of a pregnancy exceeds that of the luteal phase of the ovarian cycle. The conceptus within the uterus, therefore, is believed to produce a substance or substances which directly or indirectly prolong the lifespan of the corpus luteum and prevent a return to ovarian cyclicity. This phenomenon is known as maternal recognition of pregnancy¹. The active substance implicated in signalling maternal recognition of pregnancy in the sheep is an embryonic secretory protein, known as ovine trophoblast protein-1 (oTP-1) (refs 2-4), which is targeted in a paracrine manner to the uterine epithelium of the mother⁵. We report here the primary amino-acid sequence of oTP-1 as inferred from a cloned complementary DNA and demonstrate that the protein is most probably an interferon- α .

Ovine trophoblast protein-1 (oTP-1) is the major secretory product synthesized by the sheep conceptus between days 13 and 21 of pregnancy^{3,6}. The protein consists of 3-4 isoelectric variants (pI 5.5-5.8) of relative molecular mass (M_r) ~18,000 (18K) (Fig. 1a).

For cell-free translation experiments, polyadenylated RNA was purified from day-16 embryonic cellular total RNA^{7,8}. The oTP-1 obtained from *in vitro* translation⁹ (pre-oTP-1) consisted of several polypeptides, each of $\sim M_r$ 21K (ref. 6) (Fig. 1b). These results indicated that oTP-1 messenger RNA was an abundant component of the poly(A)⁺ mRNA and that several distinct oTP-1 mRNAs were probably present. Poly(A)⁺ mRNA was annealed to an oligo(dT) primer and used to construct a cDNA library in bacteriophage λ gt11 (refs 10, 11). Clones containing cDNA for oTP-1 were identified by screening¹⁰ the library with a rabbit antiserum raised against oTP-1 (ref. 5). Five positive isolates were plaque-purified, and two, termed kaz-2 of 550 base pairs (bp) and kaz-3 of 350 bp in length, were sequenced after subcloning into the *EcoRI* site of the pUC13 plasmid¹². Plaques were tested for the presence of a β -galactosidase fusion protein¹³. Of 12 different positive isolates, the largest fusion protein (M_r 126K) was derived from kaz 2. None of the recombinant phage isolated from the λ cDNA library contained a cDNA specifying a full-length polypeptide. ³²P-

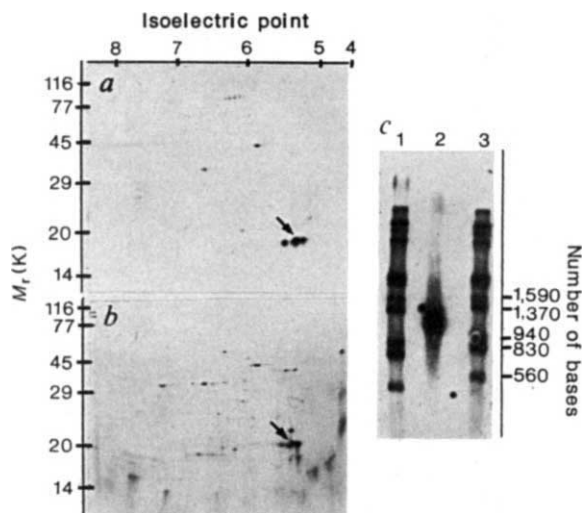


Fig. 1 *a*, Two-dimensional polyacrylamide gel electrophoresis of polypeptides released by an intact day 16 sheep conceptus after *in vitro* culture. Conceptuses were flushed from the uterus, cultured in presence of L-[³H] leucine, and the medium analysed as described³. Radioactive polypeptides were detected by fluorography of the dried gel. The 3–4 isoforms of oTP-1 are arrowed. *b*, Analysis of the *in vitro* translation products of day 15–17 sheep conceptus mRNA. About 2.5 µg of poly(A)⁺ mRNA was translated in presence of L-[³⁵S]methionine and analysed as above⁶. The polypeptides that can be immunoprecipitated by anti-oTP-1 antiserum are arrowed. *c*, Northern blot analysis of poly(A)⁺ mRNA isolated from day 16 sheep conceptuses (lane 2). Electrophoresis was carried out in a 1.5% agarose-formaldehyde gel and the RNA blotted on to nitrocellulose¹⁵. The detecting probe was ³²P-labelled kaz-2. Radioactivity on the dried gel was detected by autoradiography. The standards (lanes 1 and 3) were ³²P-labelled γ DNA cut with *Eco*RI and *Hind*III.

labelled kaz-2 was used to determine the size of oTP-1 mRNA by Northern blot analysis. The length of the poly(A)⁺ mRNA was calculated to be about 1.1 kilobases (kb) (Fig. 1c). As clone kaz-2 contained the 3' end of the mRNA, we used an 18-mer synthetic oligonucleotide d(GTAACTTAACAAATTTC) which represented the complement of a sequence starting 37 bases from the 3' end as a primer to construct a new cDNA library from the original poly(A)⁺ mRNA in the plasmid pBR322¹⁴. This library was screened for oTP-1 inserts using ³²P-labelled kaz-2 as a probe^{14,15} when several new clones encoding oTP-1 were identified, the largest of which (kaz-6) was 918 bp long.

The cDNA nucleotide sequence shown in Fig. 2 derives from clone kaz-6, except for the 54 bases at the 3' end, which are from kaz-2. The sequence is 972 bases long up to the beginning of the poly(A)⁺ tail. It contains an open-reading frame of 195 codons which begins with an ATG codon 80 bases from the 5' end and ends in a termination codon at base pair 666. The primary amino-acid sequence of oTP-1 inferred from this nucleotide sequence has 45–55% sequence homology with a range of human^{16–18}, bovine^{18,19}, mouse²⁰, rat²¹ and pig²² interferons (IFNs) of the alpha family. These interferons themselves have only a low degree of sequence identity; their homology with oTP-1 therefore must be significant. The most extensive sequence similarity of oTP-1 is to bovine IFN- α_{II} (70.3%)¹⁸. No other known protein sequence was homologous with oTP-1. Like most pre-IFNs, oTP-1 has a 23-residue signal sequence and a conserved Ser-Leu-Gly preceding the position-1 cysteine. Several amino-acid residues which are found in most IFN- α s so far characterized are conserved in oTP-1. These include cysteines at positions 1, 29, 99 and 139 and the Cys-Ala-Trp-Glu-Ile-Val-Arg-Val sequence (residues 139–146) within the highly

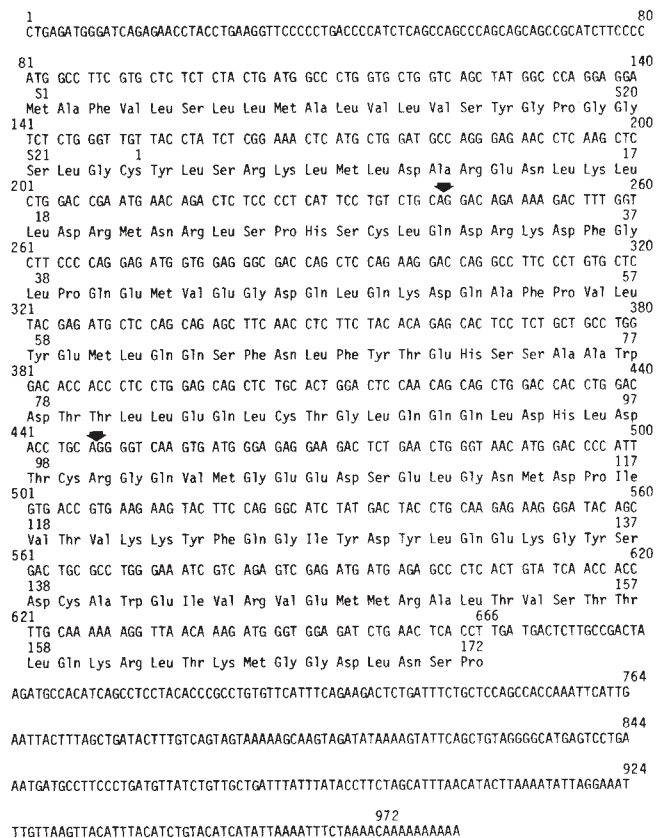


Fig. 2 Nucleotide sequence and deduced amino-acid sequence of the full length oTP-1 cDNA from clones kaz-6 and kaz-2. The predicted amino-acid sequence is given below the nucleotide sequence. The probable signal sequence is numbered s1 to s23 and the mature protein 1 to 172. The potential polyadenylation signal is underlined and the two *Pst*I sites are arrowed.

Methods. The entire 918-bp of clone kaz-6 was sequenced directly out of pBR322¹⁴ in both orientations. To confirm the sequence in the middle of the cDNA, a 21-mer oligonucleotide 5'-d(GGGTGTAGGAGGCTGATGTGG)3', the complement of bases 690 to 710, was used as a primer for sequencing of bases 689 to 380. The sequence from base 102 to 918 has also been confirmed in a second clone (kaz-5) which differed from kaz-6 only at bases 162, 163 and 166 (G, A and G respectively).

conserved 118–146 cluster. Similarities to IFN- α s also occur throughout the oTP-1 sequence. Confirmation that the cDNA shown in Fig. 2 corresponds to oTP-1 was provided by N-terminal amino-acid sequencing of purified oTP-1, through Western blotting of the β -galactosidase fusion proteins, and by epitope selection experiments²³.

Interferons were originally described as antiviral proteins, but it is clear that they have a wide range of activities^{24,25}. There are numerous IFN- α genes in all animal species so far examined, and the oTP-1 isoforms might represent IFNs of highly specialized function. Further, as a group of proteins immunologically related to oTP-1 is also produced by bovine conceptuses²⁶ at an equivalent period of their development, IFNs may be implicated in maternal recognition of pregnancy in species other than sheep. A series of cDNA clones corresponding to this bovine protein (bTP-1) have recently been sequenced (K.I. and R.M.R., unpublished results), and the inferred primary structure of bTP-1 also indicates that it could be an interferon.

Finally, IFNs can delay allograft rejection and inhibit lymphocyte activation by lectins and antigens^{24,25}. It is plausible, therefore, that oTP-1 might also participate in the immune protection of the fetal allograft.

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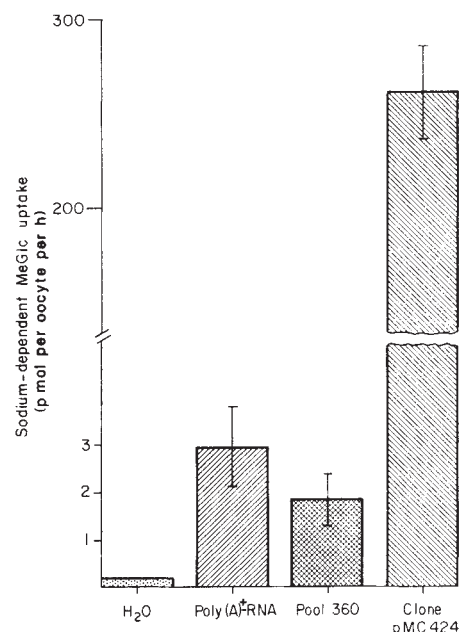


Fig. 1 Sugar uptakes at the initial and final stages of screening. Oocytes were injected with H₂O, rabbit intestinal poly(A)⁺-RNA, and RNA synthesized from clones. Poly(A)⁺-RNA from intestinal mucosa was fractionated by preparative agarose gel electrophoresis as described previously¹¹. Aliquots of fractionated mRNA were injected into *Xenopus laevis* oocytes, and the transporter was assayed by measuring the Na⁺-dependent uptake of ¹⁴C- α -methyl-D-glucopyranoside¹¹. The mRNA encoding the Na⁺/glucose co-transporter was found in a single fraction (see Fig. 2 in ref. 11). This fraction was used to prepare a cDNA library using an *in vitro* expression vector. Synthetic mRNA was prepared from the clones and injected into oocytes.

Methods: cDNA was synthesized by the method of Gubler and Hoffman³⁰, and *Eco*RI linkers were added. Full size cDNA was separated from partial products by electrophoresis using the preparative electrophoresis apparatus³¹. The material was ethanol precipitated and ligated into the Bluescript *in vitro* expression vector (Stratagene). About 2,000 clones were obtained. Synthetic mRNA was prepared from the clones using a modification of the method of Krieg and Melton³². Plasmid DNA was extracted by a mini-prep technique³³ followed by RNase A treatment, and the plasmid DNA linearized with *Not* I and then used for *in vitro* transcription and capping. T3 polymerase (Stratagene) was used, and the procedures were as specified by the manufacturer. RNA was purified by one phenol/chloroform extraction. Unincorporated ribonucleotides and the RNA cap structure were removed by two ethanol precipitations. The RNA (~4 μ g) was resuspended and assayed using the oocyte expression assay. Initially RNA was prepared from groups of about 300-500 clones and tested using the expression assay. One of the groups was positive. The group was subsequently subdivided until a single clone was isolated. RNA (50 nl; 0.2-0.3 μ g μ l⁻¹) was injected into oocytes, and uptakes were assayed 3 days later. Uptakes are expressed as the increase in Na⁺-dependent α -methyl-D-glucopyranoside uptake induced 3 days after RNA injection. Uptakes were measured in 5-6 cells in the presence and absence of Na⁺. Na⁺-dependent uptake is given as the mean $\pm$ s.e.m.

Expression cloning and cDNA sequencing of the Na⁺/glucose co-transporter

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Organic substrates (sugars, amino acids, carboxylic acids and neurotransmitters) are actively transported into eukaryotic cells by Na⁺ co-transport. Some of the transport proteins have been identified—for example, intestinal brush border Na⁺/glucose and Na⁺/proline transporters^{1,2} and the brain Na⁺/Cl⁻/GABA transporter³—and progress has been made in locating their active sites and probing their conformational states^{1,2,4-7}. The archetypical Na⁺-driven transporter is the intestinal brush border Na⁺/glucose co-transporter (see ref. 8), and a defect in the co-transporter is the origin of the congenital glucose-galactose malabsorption syndrome⁹. Here we describe cloning of this co-transporter by a method new to membrane proteins. We have sequenced the cloned DNA and have found no homology between the Na⁺/glucose co-transporter and either the mammalian facilitated glucose carrier or the bacterial sugar transport proteins. This suggests that the mammalian Na⁺-driven transporter has no evolutionary relationship to the other sugar transporters.

Traditional methods for cloning membrane proteins require either antibodies or synthetic oligonucleotide probes derived from partial amino-acid sequences to screen cDNA libraries.

None of the mammalian co-transport proteins have been cloned, partly because of difficulties in the purification of low abundance hydrophobic membrane proteins (<0.2% of the membrane protein). We report here the successful application of a new strategy to clone the rabbit intestinal Na⁺/glucose co-transporter without antibodies or oligonucleotide probes. Our strategy parallels the novel approach used to clone interleukin 4 (ref. 10) and has been developed from two observations: (1) injection of poly(A)⁺-RNA from the rabbit intestine into *Xenopus laevis* oocytes resulted in the expression of Na⁺-dependent, phlorizin-sensitive α -methyl-D-glucopyranoside uptake into oocytes; and (2) after size-selection of poly(A)⁺-RNA by electrophoresis, the

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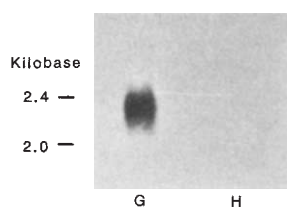


Fig. 2 Northern blot of rabbit jejunal (G) and heart ventricular muscle (H) mRNA.

Methods. 5.5 µg of poly(A⁺)-RNA samples and 2.5 µg of RNA standards (BRL) were electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde and electroblotted. Electrophoresis and electroblotting were performed in 40 mM MOPS, 10 mM Na acetate, 1 mM EDTA, pH 7.0. Hybridization was performed for 24 h at 65 °C as described by Church and Gilbert³⁴ with the exception that the hybridization solution contained 50% formamide and 250 mM Na phosphate buffer at pH 7.2; washings were performed at 65 °C using an OmniBlot apparatus (ABN). The hybridization probe was antisense RNA transcribed from clone pMC424 as described by the supplier (Stratagene) using T7 polymerase.

mRNA coding for the co-transporter was found in a 2.3-kilobase (kb) fraction¹¹. Complementary DNA was synthesized from this enriched fraction and the full-length cDNA was isolated by preparative electrophoresis. A plasmid library was constructed from the cDNA in a transcription vector. The colonies were screened by injecting mRNA synthesized *in vitro* into oocytes and assaying for Na⁺-dependent ¹⁴C-α-methyl-D-glucopyranoside uptake. A single clone (pMC424) was isolated, and RNA synthesized from this clone increased Na⁺-dependent sugar uptake more than 1,000-fold (Fig. 1), but it had no effect on the rate of Na⁺-independent uptake. Sugar analogues inhibited α-methyl-D-glucopyranoside uptake in the sequence expected for the intestinal brush border Na⁺/glucose co-transporter; that is, D-glucose > α-methyl-D-glucoside > D-galactose > 3-O-methyl-D-glucopyranose > L-glucose. Phlorizin blocked uptake, and Na⁺-activated uptake with a Hill coefficient of 1.5. These results establish that we have cloned the cDNA for the 'classical' intestinal brush border Na⁺/glucose co-transporter⁸ rather than another glucose transporter. Anti-sense RNA synthesized from the clone hybridized with a 2.3-kb band of intestinal mRNA but did not hybridize with heart mRNA (Fig. 2). This is consistent with the tissue distribution of the co-transporter, and as the heart does contain mRNA coding for the facilitated glucose carrier¹², it also indicates little homology between the two mammalian glucose carriers.

The DNA sequence and the deduced amino-acid sequence of the Na⁺/glucose co-transporter are presented in Fig. 3. The size of the clone, 2,225 base pairs (bp), is compatible with mRNA identified by Northern analysis, and this indicates we have isolated a virtually full-length clone. The translation initiation site is at the first ATG codon as it lies within the consensus initiation sequence CCGCCATGG¹³, and the open reading frame continues to the first stop codon TGA at base 2,010. This open frame codes for 662 amino-acid residues with a relative molecular mass (*M_r*) of 73,080 which is consistent with the *M_r* of the brush border Na⁺/glucose co-transporter (75,000)⁴. We have not yet eliminated the possibility that the synthetic RNA injected into oocytes activates endogenous Na⁺/glucose transport. This is highly unlikely in view of the magnitude of the increase in transport (>1,000-fold) and the agreement between the predicted and measured sizes of the transport protein.

The hydrophobicity plot for our predicted amino-acid sequence and our proposed model are shown in Fig. 4. The membrane-associated α-helices were determined by hydrophobic moment analysis¹⁴, and the secondary structure of the hydrophilic sequences was assigned by the Garnier method¹⁵. The model contains 11 membrane-spanning sequences. Five of

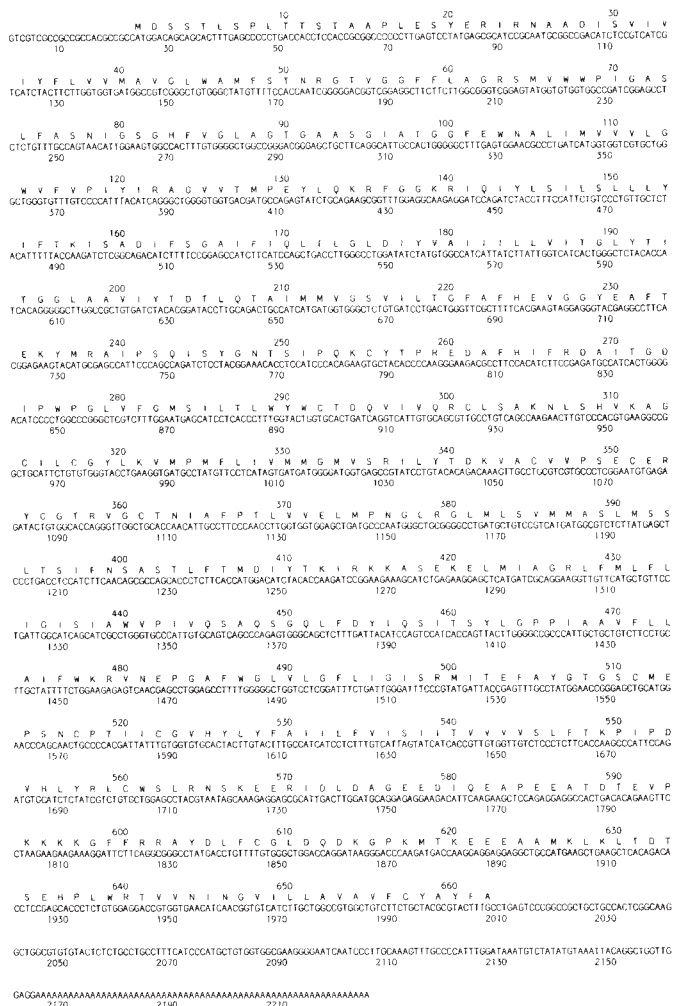


Fig. 3 Nucleotide sequence of cDNA and deduced amino-acid sequence of Na⁺/glucose co-transporter. The primary amino-acid sequence was deduced from a codon preference plot and from the largest single open reading frame.

Methods. Plasmid DNA from the clone was extracted and purified by electrophoresis as described earlier³¹. The DNA was cleaved with *EcoRI* and the 2.2-kb insert was purified by electrophoresis. The whole insert, as well as sequencing fragments prepared by restriction enzymes, was cloned into M13mp18 and 19. The DNA was sequenced according to Sanger³⁵ with modifications as described previously³⁶. The poly-A tail region was sequenced from the 3' end using the DNA polymerase 'Sequenase' (US Biochemical Corp.) according to the manufacturer's instruction. Oligonucleotides were synthesized by Dr Dohn Glitz (UCLA Medical Center) and used as primers for further sequencing.

these (2, 5–7 and 9) are amphipathic and may interact to stabilize the structure by forming interhelical H-bonds and salt bridges. A feature of the hydrophilic domains is the presence of two long, highly polar regions near the C terminus. One links membrane spans 10 and 11 and contains 40 charged residues in three concentrated clusters: one of 20 amino acids containing 10 acidic residues; a second of 10 amino acids containing six basic residues; and a third containing three glutamic acids. The second large polar domain is on the opposite side of the membrane between spans 7 and 8 and contains 17 acidic and basic residues. Five of the basic residues are within a short stretch of nine amino acids. Two shorter hydrophilic segments, one on each side of the membrane, contain potential N-linked glycosylation sites. We speculate that the two highly charged hydrophilic segments are involved in glucose binding. Previous work indicates multiple lysine residues at or near the glucose binding site located in a cleft in the protein^{4,7}. In sugar-binding proteins¹⁶,

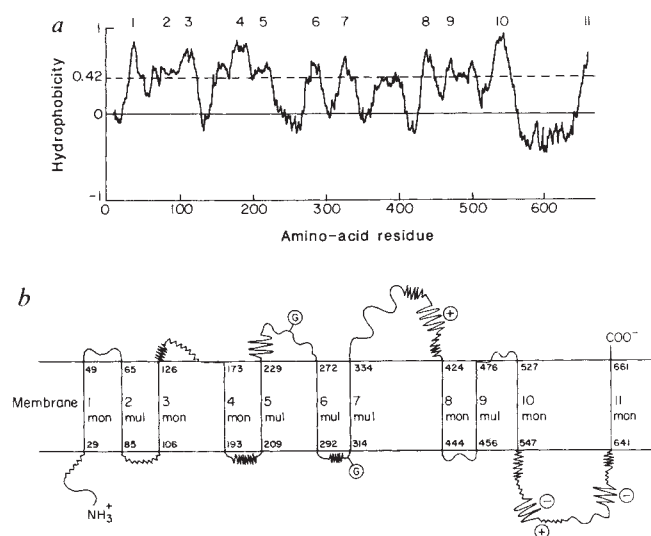


Fig. 4 *a*, Hydrophobicity plot of the Na⁺/glucose co-transporter amino-acid sequence. The method of Eisenberg *et al.*¹⁴ was used with a 21 amino-acid window, and the numbers 1 to 11 refer to postulated membrane-spanning segments. *b*, Proposed model for the orientation of the Na⁺/glucose co-transporter in the membrane. The transmembrane helices were detected and analysed according to the method of Eisenberg *et al.*¹⁴. Depending on the hydrophobic moment, the segments are labelled either monomer (mon), or multimer (mul). The structure of the non-transmembrane regions was examined according to secondary structure predictions of Garnier *et al.*¹⁵. G indicates N-X-T/S sequences which represent potential N-linked glycosylation sites. The secondary structure elements are indicated as follows: $\sim$, B-turn; --- , β -sheet; W , α -helix; and --- , random. Clusters of negative and positive charges are represented as $\ominus$ and $\oplus$.

the sugar binding site is located deep in a cleft between a bilobular structure, and extensive use is made of charged residues to form H-bonds to the sugar. Tertiary structure analysis¹⁷ of the arabinose binding protein at 1.7 Å resolution shows H-bonding of the sugar to Lys, Arg, Glu, Asp, and two Asn residues.

Previous studies provided evidence that a tyrosine residue is located at, or near, the Na⁺-binding site⁵ and that the Na⁺ active site is 30–40 Å away from the glucose site⁶. Na⁺ binding to the transporter produces a long-range conformational change that increases the affinity of the glucose site for substrates^{7,8}. There are 27 tyrosine residues in the protein, but our model provides no clues about the location of the tyrosine at the Na⁺-binding site.

Several functionally related transport proteins, the mammalian facilitated glucose carrier^{18,19} and *Escherichia coli* sugar co-transporters^{20–23}, have been cloned and sequenced. There are extensive sequence homologies between the mammalian glucose carrier and the *E. coli* arabinose and xylose H⁺-co-transporters²¹. Unexpectedly, there is no detectable homology between the Na⁺ glucose co-transporter and either the facilitated glucose carrier or the *E. coli* sugar transporters. The Diagon plots²⁴ show no homologous regions (21-residue window and a 240 score limit), and there is no hybridization of anti-sense RNA from pMC424 to heart 2.6–2.8-kb mRNA (Fig. 2). This is consistent with the inability of red cell glucose carrier antibodies to react with the brush border Na⁺/glucose co-transporter²⁵. In addition, the Na⁺ glucose co-transporter does not contain the motif $\text{---R}^{\text{RR}}\text{XG}^{\text{RR}}\text{KK}^{\text{RR}}\text{---}$, which is highly conserved in the facilitated glucose carrier and in the *E. coli* H⁺ and Na⁺ co-transporters²¹. Comparison of the Na⁺/glucose cotransporter, DNA and amino-acid sequences with those in GenBank and NBRF Protein Identification Resource files also shows no homology with other sequences. This suggests that the Na⁺/glucose co-transporter

may represent a novel class of transport proteins distinct from channels^{26,27} and pumps^{28,29}.

The strategy used to isolate the clone for the intestinal Na⁺/glucose co-transporter may prove useful in the isolation of clones for other membrane proteins. In cases where it is difficult to isolate rare membrane proteins and/or to make antibodies, our approach requires only a sensitive functional assay for the protein in an expression system such as oocytes.

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Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein

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Synthetic vaccines for viral diseases can use defined regions of viral proteins as immunogens^{1,2}: the peptide sequence of amino acids 141–160 of the VP1 protein of foot and mouth disease virus (FMDV) elicits virus-neutralizing antibodies to protect guinea pigs, cattle and pigs either when coupled to a carrier protein or when administered in liposomes or in incomplete Freund's adjuvant^{3–5}. The immune response to these peptides is much lower than that to complete virus particles¹ and the same sequence fused

to the N terminus of β -galactosidase did not produce a more potent immunogen than synthetic peptide alone⁶. We report here an expression system for immunogenic epitopes linked to a carrier protein, hepatitis B core antigen, to form part of a virus-like complex which can present these epitopes to the immune system at high density. The immunogenicity of these structures approaches that of FMDV particles.

The core protein of hepatitis B virus (HBV) self-assembles into 27-nm particles when expressed in *Escherichia coli* cells. These stable particles can be purified⁷ and visualized in the electron microscope⁸, and are highly immunogenic in laboratory animals. The core antigen (HBcAg) has an arginine-rich region towards the carboxy terminus which has some homology with a protamine sequence and which could form a nucleic-acid binding domain. A soluble, partially degraded form of the core protein (HBcAg) with different antigenic properties is found in the serum of HBV carriers; this truncated protein lacks only the arginine rich C-terminal region and does not form core particles⁹. It is inferred that the arginine-rich sequence bound to viral nucleic acid is critical to core particle assembly¹⁰. We have constructed expression plasmids in which the main immunogenic region of FMDV (amino acids 142–160 of virus protein VP1, serotype O₁, Kaufbeuren strain) was fused to the amino terminus of the core antigen, thereby avoiding interference with particle assembly.

The 5' end of the HBcAg gene transcript has two in-frame initiation codons, the first of which initiates a pre-core signal peptide and leads to secretion of the core protein from the cell as HBcAg (refs 11–14). Our initial constructs expressed a fusion protein in bacteria with the signal sequence replaced by the FMDV epitope but this was toxic to *E. coli* and no expressed protein was detectable (results not shown). We therefore transferred the fusion gene into a vaccinia virus (VV) shuttle vector (Fig. 1) under the control of the VV p11k promoter¹⁵. Translation from this fusion gene transcript should initiate at the authentic p11k AUG codon to give a short linker sequence followed by the main immunogenic site from FMDV, six amino acids of pre-core, and the entire core protein. Transfection of this plasmid into VV-infected CV1 cells gave a number of plaques negative for thymidine kinase (TK⁻) and containing FMDV/HBcAg DNA, as determined by DNA hybridization (not shown).

Cell lysates from recombinant VV-infected cells were tested for core and FMDV antigens using a sandwich enzyme-linked immunosorbent assay (ELISA). As can be seen in Fig. 2a, a protein from infected cell lysates could be trapped with FMDV 142–160 anti-peptide serum or with anti-virus particle serum and could then be recognized by either anti-core, anti-peptide or anti-virus particle serum in the second phase. This protein was purified by ultracentrifugation followed by sucrose density gradient sedimentation and fractions were assayed for core

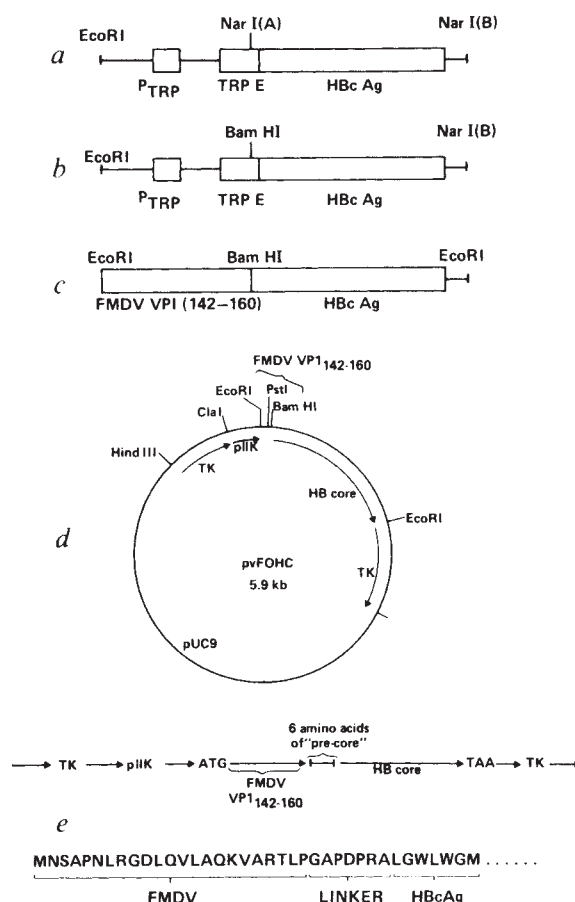


Fig. 1 The construction of the shuttle vector used to introduce the FMDV/HBcAg fusion gene into the genome of vaccinia virus. *a*, Plasmid clones containing HBV sequences (adw serotype) representing the gene coding for the core antigen were engineered by *Bal31* digestion so that the core sequence was fused to the first 15 amino acids of the *E. coli trp E* gene. *b*, This plasmid was partially digested with *NarI* and a *Bam*HI linker inserted at *NarI*(A). *c*, The *Eco*RI-*Bam*HI fragment from the resulting plasmid was then replaced with a synthetic oligonucleotide linker coding for amino acids 142–160 of VP1 from FMDV, serotype O₁ (Kaufbeuren) and an *Eco*RI linker insertion at *NarI*(B). *d*, This construct was subcloned into a vaccinia virus (VV) shuttle vector¹² as an *Eco*RI fragment. This plasmid pVFOHC was introduced into the Wyeth strain of VV by homologous recombination through the flanking thymidine kinase (TK) sequences to produce recombinant virus vFOHC. VV methodology was performed as described¹⁸. *e*, Amino acid sequence of the amino-terminus of the hybrid protein.

Table 1 Immune response of guinea pigs to a fusion protein of hepatitis B core antigen with FMDV 142–160 peptide

Core fusion protein	Dose (μ g) FMDV component	Antibody assay	Days post-inoculation					Number of animals protected
			0	7	14	28	42	
2	0.2	Anti-142–160 cys peptide ($\log_{10}$)	<1.0	<1.0	1.6	2.7	3.2	3.1
		Mean neutralizing antibody ($\log_{10}$ SN ₅₀)	<0.6	<0.6	1.2	2.1 (1.3–2.6)	2.9 (2.0–3.3)	2.9 (2.0–3.3)

The immunogenicity of the fusion protein was assessed by inoculating each of four guinea pigs intramuscularly with a 2 μ g dose, containing 0.2 μ g of FMDV peptide sequence and formulated as a water-in-oil emulsion. Before inoculation each preparation of chimaeric particles was passed through 0.45 μ m nitrocellulose filters and the filtrate tested for complete removal of live recombinant vaccinia virus. Serum samples were collected at regular intervals for 56 d then the immune status of the animal was challenged by intradermal inoculation of 500 guinea pig ID₅₀ O₁ Kaufbeuren virus. The neutralizing activity of serum samples against 100TCID₅₀ of virus was determined using a micro-neutralization test with IBRS2 cells¹⁹. Anti-peptide titres were obtained using an indirect ELISA assay with synthetic peptide (142–160 cys) bound to the solid phase⁴. Log₁₀ titres were calculated by reference to the optical density of a range of negative pre-immune sera at a 1:10 dilution. Mean titres for the four animals are given. Ranges for 28 and 56 day sera are shown in brackets.

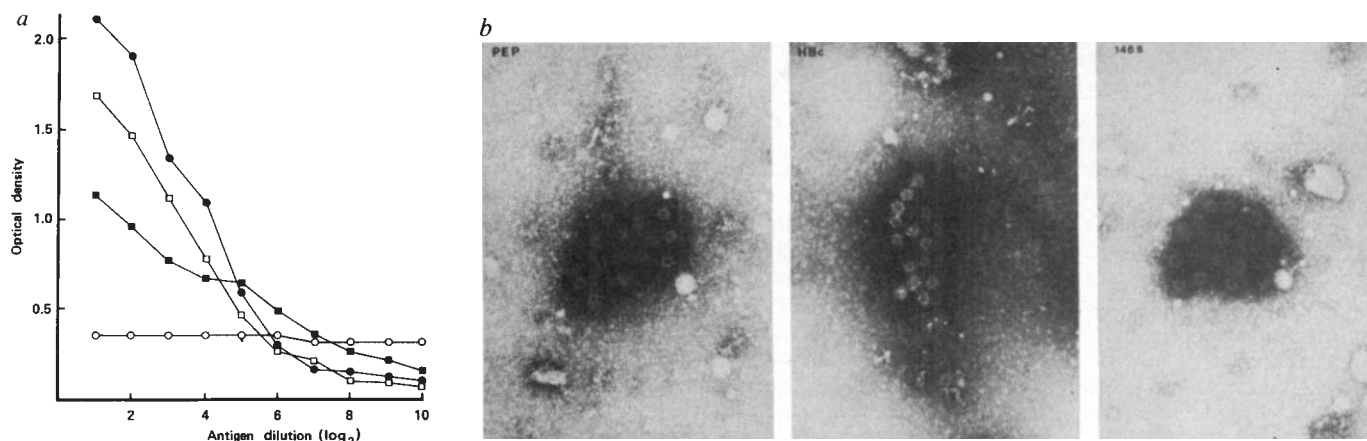


Fig. 2 *a*, Sandwich ELISA of cell lysates from (○) wild-type vaccinia control (Wyeth) or (●, □, ■) recombinant (vFOHc) infected cells. *b*, Electron micrographs of purified particles complexed with antiserum to FMDV particles (146 S), 142-160 peptide (PEP) or natural core particles (HBc). Complexes were adsorbed to form var-coated grids and negatively stained with phosphotungstic acid.

Methods. *a*, Confluent monolayers of HeLa TK⁻ cells in 175 cm² tissue culture flasks were infected at an MOI of 10. After adsorbing for 1 h at 37 °C with occasional agitation, the inoculum (1 ml) was diluted with 20 ml Eagle's medium containing 2% fetal calf serum. After incubation for 24 h at 37 °C the infected cells were shaken off the flasks and recovered by centrifugation. The cell pellet was resuspended in 20 ml of Eagle's medium and after three cycles of freezing and thawing the disrupted cells were clarified at 3,000 r.p.m. and then 15,000 r.p.m. for 30 min in an SS34 rotor. Antigen from infected cells was then bound to ELISA plates using either (■) FMDV particle (146 S) or (●, ○, □) FMDV VP1 142-160 antisera raised in rabbits. Each trapped antigen was then assessed for the presence of either (●, ○) HBc, (■) FMDV 146S or (□) FMDV 142-160 epitopes by binding with the respective guinea pig antisera and development with anti-guinea pig peroxidase conjugate. *b*, Hybrid core particles prepared as described in *a* were collected by centrifugation at 55,000 r.p.m. for 3 h in a Ti70 rotor and separated on 15-45% sucrose gradients at 28,000 r.p.m. in an SW28 rotor for 4 h. Core particles sedimented to the middle of the gradient and could be located by ELISA. Peak fractions were pooled and immune complexes prepared.

antigen by ELISA: a peak of HBcAg reactivity was detected sedimenting at the same position as native core particles expressed in bacteria and run on parallel gradients. Thus the FMDV sequence does not apparently interfere with the assembly of the core particles.

The ability of the FMDV/HBcAg fusion protein to self-assemble into regular 27 nm core-like particles was confirmed by examination in the electron microscope of immune complexes formed with sucrose gradient-purified material. The complexes are shown in Fig. 2*b* and were formed by reacting the particles with antiserum to intact FMDV particles, to 141-160 peptide or to natural core particles. Interestingly, the complexes formed with any anti-FMDV sera had more space between the particles than was found between anti-core serum complexes, suggesting that FMDV reactive epitopes protrude from the particle surface and are thus accessible to the immune system. The reaction of the particles with anti-FMDV sera indicates that the FMDV sequences can adopt a virus-like conformation on the surface of the particle. Furthermore, ELISA consistently showed greater reaction of particles with anti-virion compared with anti-peptide sera.

The immunogenicity of the FMDV/HBcAg fusion protein

was assessed in guinea pigs: after inoculation with 2 µg of protein all animals developed significant amounts of anti-peptide and virus-neutralizing antibodies within 14 days (Table 1). This antibody activity reached a plateau of ~3.0 log₁₀ at 42 days and gave complete protection against virus challenge at 56 days. Therefore as little as 0.2 µg FMDV 142-160 peptide corresponding to 10% of the fusion protein, presented on the surface of core particles gave full protection to guinea pigs. A further group of guinea pigs, inoculated with only 0.2 µg chimaeric particles (0.02 µg of FMDV peptide), did not produce a primary neutralizing antibody response. However, boosting with a further 0.2 µg fusion protein elicited an anamnestic response. Immunological priming has been demonstrated before with FMDV synthetic peptides^{3,4} but only using much greater quantities of antigen.

Table 2 compares the immunogenicity of inactivated FMDV particles, serotype O₁, with a range of synthetic and biosynthetic antigens including the FMDV/HBcAg fusion protein. On a weight for weight basis, 142-160 peptide on core particles is ~30-40 times more immunogenic than peptide/β-galactosidase fusion protein containing tandem copies of the FMDV epitope¹⁷, 500 times more immunogenic than free synthetic peptide³, and

Table 2 Comparative immunogenicity of inactivated FMDV particles, serotype O₁, with a variety of synthetic and biosynthetic antigens

Test sample	Total antigen†	Dose (µg) FMDV VP1 142-160 sequence	Days post-inoculation		
			0	28	42
Inactivated FMDV particles	1	0.02	<0.6*	1.6	1.7
HBcAg/142-160 fusion protein	2	0.2	<0.6	2.1	2.9
β-gal/(137-162) ₂ fusion protein	200	7	<0.6	1.5	1.9
142-160 cys peptide	100	100	<0.6	1.8	1.7
137-160 cys peptide	120	100	<0.6	2.7	2.2

* log₁₀ SN₅₀ against 100 TCID₅₀ of virus¹⁹ (neutralizing antibody).

† These doses are the minimum required to elicit ~2 log of neutralizing antibody.

The immunogenicity of inactivated FMDV particles in guinea pigs (M.J.F., unpublished data) has been compared with that of the HBcAg/FMDV 142-160 fusion protein, and with other published results for β-galactosidase/FMDV (137-162)₂ fusion protein¹⁷, uncoupled 142-160 cys peptide³ and 137-160 cys peptide³. All antigens were inoculated intramuscularly using incomplete Freund's adjuvant.

almost as immunogenic as inactivated virus particles. This is the best response so far reported for any subunit immunogen of FMDV.

Particulate structures have been used previously for the presentation of antigenic epitopes to the immune system with hepatitis B surface antigen, which also forms subviral particles, acting as the carrier protein and having an immunogenic epitope of poliovirus inserted into the structure¹⁶. The immune response in mice was extremely poor, even after two injections¹⁶, but was rather better in rabbits (R. Crainic, personal communication). This contrasts with our results after one injection of the FMDV core antigen but, without a direct comparison of the poliovirus epitope in the core system, it is difficult to explain the difference between the responses. Possibly the poliovirus epitope is not located in an immunologically dominant region of the surface antigen particle or the core antigen itself could be providing antigenic determinants for critical stimulation of T-cell responses²⁰. The greater response in the core system could result from the increased epitope density; multiple copies of the FMDV immunogenic site fused to β -galactosidase give a superior response if tandem repeats of the FMDV sequence are used rather than a single copy¹⁷. It will be interesting to determine whether double copies of the FMDV sequence in the core antigen can further improve the immunogenicity of the particle. Another advantage of the core system is that additional epitopes are on the surface of the particle and hence do not contribute to its structure, thus potentially permitting any peptide sequence to be expressed in this way. Indeed, hybrid core particles could possibly express different epitopes, thus providing immunity to several virus strains in one vaccine. An important factor in considering the hepatitis B core system as a prospective vaccine is dependence of particle assembly on interaction with nucleic acid. Whereas bacterially expressed particles do contain nucleic

acid we have not so far detected any in a similar mass of the hybrid particles.

Expression of immunogenic epitopes on the surface of particles has recently been reported using the TYA gene from yeast²¹, although the immunogenicity of these particles was disappointingly low compared with the results presented here; this could reflect a stimulation by the HBcAg of T-helper cell activity²² to potentiate antibody responses against the foreign epitope.

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Characterization of β -thalassaemia mutations using direct genomic sequencing of amplified single copy DNA

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Direct sequencing of specific regions of genomic DNA¹ became feasible with the invention of the polymerase chain reaction (PCR) which permits amplification of specific regions of DNA²⁻⁵. Recently, human mitochondrial DNA was amplified and directly sequenced⁶. Using a thermostable DNA polymerase of *T. aquaticus* (Saiki, R. K. *et al.*, manuscript in preparation) in the PCR, we have applied a combination of PCR and direct sequence analysis of the amplified product to a human single-copy gene. We studied the genomic DNA of five patients with β -thalassaemia whose mutant alleles were uncharacterized, and found two previously undescribed mutations, along with three known alleles. One new allele is a frameshift at codons 106-107 and the other is an A-C transversion at the cap site (+1) of the β -globin gene. This latter is the first natural mutation observed at the cap site and it occurs in a gene which is poorly expressed.

Molecular characterization of β -thalassaemia is nearly complete. In recent years, cloning, subcloning, and sequencing of mutant β -globin genes has rarely resulted in the discovery of new alleles⁶. We have developed a method for direct sequencing

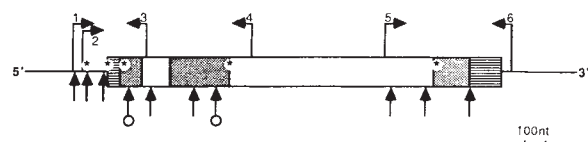


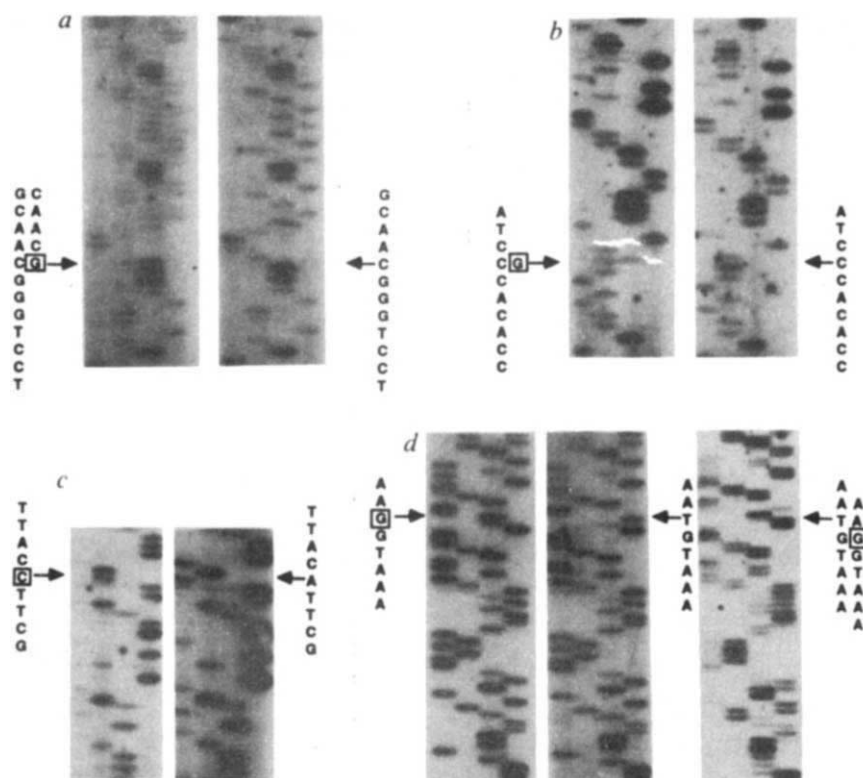
Fig. 1 Diagrammatic representation of the β -globin gene with locations and directions of PCR primers indicated by arrows above bar. For all individuals, PCR primer pairs 2 and 4 (727 nucleotides (nt)) and 5 and 6 (563 nt) were used in amplification. Primer pair 1 and 3 (336 nt) was used to study the distal promoter region from the Greek individual. Arrows below the gene represent the location of primers used to sequence in the forward direction and arrows with open circles represent the location of primers used for reverse direction sequencing. The position of each β -thalassaemia mutation found is marked by an asterisk within the gene.

of single copy genes in human genomic DNA, using a combination of *in vitro* target amplification followed by sequence analysis of amplified product, as reported for examining variation in human mitochondrial DNA⁷. In humans there are 10^3 - 10^4 copies of mitochondrial DNA, however, and single-copy genes account for only two copies per cell. In this study, we performed direct sequence analysis on the single-copy β -globin gene after amplification of genomic DNA by *Taq* DNA polymerase.

Known β -thalassaemia alleles and β^S genes were amplified and sequenced using the PCR and sequencing primers shown in Fig. 1. Nucleotide substitutions, such as those producing the β^{39} nonsense codon, β^{17} nonsense codon, and -29 A-G mutation, frameshift alleles, such as $\beta^{41,42}(-CTTT)$, $\beta^{8,9(+G)}$ and β -globin gene polymorphisms, were readily detected in both homozygotes and heterozygotes (data not shown). In heterozygotes, both normal and mutant alleles are equally amplified by PCR, and subsequent extension from any one sequencing primer occurs on both alleles. This results in the superimposition of sequencing ladders derived from the two β -globin genes.

Direct sequence analysis was then carried out on five

Fig. 2 Genomic sequencing of β -thalassaemia mutations. In each panel the mutant sequence is shown on the left and the normal sequence on the right. Lanes from left to right are A, C, G, T. *a*, American black individual, frameshift 106–107; *b*, Greek individual, C to G at –87; *c*, *d*, Asian Indian individual, A–C substitution at +1 of the β -globin gene. In *c*, sequence is shown for the father (left) and normal control (right) on the sense strand. In *d*, sequence on the anti-sense strand is shown for the father (left), control (middle) and β^+ -thalassaemic offspring (right). The thalassaemic offspring inherited the +1 mutant allele from the father and a frameshift 8–9 allele from the mother. The +1 mutation appears frameshifted in the last sequencing panel due to the frameshift 8–9 mutation located between the primer and the +1 position. The mutations are noted by the nucleotides enclosed in boxes.



Methods. Genomic DNA (500 ng) was amplified by the PCR technique for 30 cycles using *Taq* DNA polymerase and PCR primers (see Fig. 1) with 2–4 minute extensions at 70 °C, 2–4 minute denaturations at 95 °C, and primer annealing at 55 °C (Saiki, R. K. *et al.*, in preparation). Extent of amplification by this method was 10^6 – 10^7 -fold. Amplified DNA was desalted and excess dNTPs were removed by spin-dialysis on a Centricon 30 (Amicon), concentrating the volume from 1 ml to 100 μ l (see ref. 17). Sequencing primers (2 pmole), end-labelled with γ -[32 P]-ATP and T4 polynucleotide kinase, were annealed to one-tenth of the amplified β -globin DNA (0.3 pmole) by heat denaturing the strands at 95 °C for 10 min in 50 mM Tris pH 8, 50 mM KCl, 5 mM $MgCl_2$ and 10 mM DTT. The reaction (10 μ l) was spun briefly to collect the condensate, equilibrated at 37 °C for 2 min, and divided into four tubes containing 2 μ l cold dNTPs and ddNTPs (ref. 18) (A: 100 μ M dCTP, 100 μ M dGTP, 100 μ M dTTP, 10 μ M dATP, 2.5 μ M ddATP; C: 100 μ M dGTP, 100 μ M dTTP, 10 μ M dATP, 20 μ M dCTP, 5 μ M ddCTP; G: 100 μ M dCTP, 100 μ M dTTP, 10 μ M dATP, 20 μ M dGTP, 6 μ M ddGTP; T: 100 μ M dCTP, 100 μ M dGTP, 10 μ M dATP, 40 μ M dTTP, 14 μ M ddTTP). Ten units of cloned MMLV reverse transcriptase (BRL) were added to each tube and the reaction was allowed to proceed for 40 min at 37 °C. In other reactions T7 DNA polymerase (Sequenase, USB) was used with ratios of dNTP to ddNTP of 10:1. Samples were boiled for 3 min in formamide dye stop mix and subjected to electrophoresis on 8 M urea/7% acrylamide wedge gels at 57 watts for 2.5 h. Gels were fixed in 10% methanol/acetic acid, dried and exposed to X-ray film for 12–24 h. Approximately 200 bases can be resolved where the first readable base is 20 nucleotides from the 3'-end of the sequencing primer.

individuals with β -thalassaemia whose mutant alleles were unknown. These individuals were from different ethnic groups: Italian, Greek, Asian Indian, Haitian and American Black. Close correlation between haplotypes and specific ethnic mutations allows one to predict the particular mutant allele present in the vast majority of β -thalassaemia heterozygotes^{8,9}. But determination of β -globin haplotypes followed by oligonucleotide analysis for the associated mutant alleles did not reveal the expected mutation in any of the five individuals.

Following PCR-amplification of two regions of the β -globin gene in the five DNA samples, roughly 1,200 nucleotides were sequenced. Comparison of sequencing ladders from all five samples easily revealed loss, addition, or shifts in position of bands. Our sequence analysis revealed two frameshift mutations in heterozygotes. A frameshift at codon 8 due to the deletion of two A residues was observed in the Italian (data not shown). This allele has been previously described only in a Turk¹⁰. In addition, an undescribed frameshift at codons 106–107 due to insertion of a G residue was observed in the American Black (Fig. 2a).

Two known nucleotide substitutions were also detected. In the Greek, a C–G transversion at –87 in the ACACCC distal promoter region was discovered (Fig. 2b), and in the Haitian, a G–A transition at intervening sequence 2 (IVS-2), nucleotide 1 (data not shown). The first mutation has previously been observed as a rare allele only in Italians⁸, and the second has been described only in the Mediterranean basin¹¹. Occurrence of these two mutations in unexpected ethnic groups is best explained, after inspection of their associated β -globin haplotypes, by gene migration.

Unexpectedly, the Asian Indian was homozygous for a previously undescribed mutation, an A–C transversion at the cap site (+1) (Fig. 2c). His haematological picture (mean corpuscular volume = 71 fl, haemoglobin A_2 (HbA₂) = 4.1%, HbF < 2%) was entirely consistent with β -thalassaemia trait. Homozygosity for the cap site change was confirmed by sequencing through the mutation in both directions (Fig. 2c, d). Oligonucleotides specific for both normal and mutant sequences were also synthesized. Unamplified genomic DNA of the subject hybridized only to mutant oligomer and not to normal oligomer, whereas that of his offspring hybridized to both oligomers (data not shown). Furthermore, according to haplotype analysis⁸ the subject was homozygous for the β -globin gene region.

It is unlikely that the cap site mutation is a neutral allele. It has not been observed in >50 sequenced β -globin genes. It was not found after oligonucleotide analysis of 11 Asian Indian β^A genes containing the same β -gene framework (framework 3⁸) as that associated with the +1 mutation. Also, it was the only variation seen in the subject's β -globin genes of 1,200 nucleotides sequenced.

RNA transcription is initiated at the cap site. It is also the site at which a 7-methyl guanosine cap is post-transcriptionally added to prevent exonucleolytic degradation of the nascent transcript¹² and to promote efficient translation¹³. A compilation of sequences including the cap site in eukaryotic messenger RNAs indicates that in 90% of them a purine, usually A, is present at nucleotide +1 (ref. 14). But a C residue has been observed at +1 in about 5% of normal mRNAs. It is noteworthy that an A–G change at the cap site of a mouse β -globin gene results in a 50% decrease in the steady state level of β -globin

mRNA in a transient expression system¹⁵. The mechanism by which our novel mutation produces β -thalassaemia is under investigation.

Accuracy is essential with any DNA sequencing method. Ambiguities in sequence due to G/C compression or secondary structure were corrected by use of T7 DNA polymerase¹⁶. All five mutations detected in this study through direct sequencing were confirmed by oligonucleotide analysis or restriction analysis (data not shown).

The speed and quantity of data obtainable by direct genomic sequencing can obviate the need for constructing haplotypes for prenatal diagnosis of β -thalassaemia. We have used the technique to determine the β -thalassaemia alleles of a Greek couple with the trait who had no relatives residing in North America. The technique has also been applied to detect a mutation in the Factor VIII gene in a male with haemophilia A (manuscript in preparation). The major advantage of the direct sequencing technique, however, is the ability to screen rapidly a large number of genomic samples for common sequence variation and for rarer disease-producing point mutations.

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trainee in the Program in Human Genetics at The Johns Hopkins University.

Note added in proof: Direct sequence analysis of Klenow-amplified β -globin genes containing known mutations has recently been carried out (Engelke, D. R., Hoener, P. A. & Collins, F. S. *Proc. natn. Acad. Sci. U.S.A.* (in the press)).

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A new fused transcript in Philadelphia chromosome positive acute lymphocytic leukaemia

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The leukaemic cells of more than 90% of chronic myelogenous leukaemia (CML) patients and of 10% of acute lymphocytic leukaemia (ALL) patients carry the t(9;22) (q34;q11) translocation which generates the Philadelphia chromosome (Ph¹). In CML the *abl* gene is translocated from chromosome 9 to the centre of the *bcr* gene on chromosome 22 and this results in production of chimaeric *bcr-abl* RNA translated into a protein of relative molecular mass (M_r) 210,000 (210K). Our data indicate that in ALL *abl* is translocated into the 5' region of the *bcr* gene. The consequence of this is the expression of a fused transcript in which the first exon of *bcr* is linked to the second *abl* exon. This transcript encodes a 190K protein kinase.

The Ph¹ chromosome, an abnormal shortened chromosome 22, is present in the leukaemic cells of 90-95% of patients with CML¹. The main consequence of the aberration is the shift of the *abl* gene from chromosome 9 into either of two small introns at the centre of the *bcr* gene which is located at 22q11 (refs 2-4). This results in transcription of a chimaeric *bcr-abl* RNA encoding a 210K protein kinase⁵⁻⁸. The fused gene is expressed during both the chronic and acute phase of the disease⁹⁻¹¹. Around 15-25% of ALL patients carry the Ph¹-chromosome¹². In a high proportion of these patients the molecular abnormality varies from typical CML in that *abl* is translocated to chromosome 22, but the breakpoint on the latter is not within the central region of *bcr* and in one case was shown to be centromeric to it¹³. In addition, the leukaemic cells of these patients contain a

190K *abl*-encoded protein kinase, rather than the 210K protein typical of CML¹⁴⁻¹⁶.

Making the assumption that the 190K protein is coded by a new chimaeric *abl* messenger RNA we attempted to obtain a complementary DNA copy of this RNA. RNA from the ALL-1 cell line derived from a Ph¹-positive ALL patient¹³ was used to construct a cDNA library in λ gt10 vector¹⁷. Some 2×10^5 plaques were screened with a radiolabelled *Bgl*II-*Hinc*II cDNA fragment containing *abl* exon II sequences⁶, and with a probe composed of a mixture of *abl* exon Ia and Ib sequences¹⁸. Two plaques (λ 7 and λ 8), which reacted with the first but not with the second probe, were purified and analysed. The clones had very similar structures and the physical map of λ 7 is shown in Fig. 1A. Sequence analysis indicated on open-reading frame extending to the 3' end of the molecule (Fig. 1B). Inspection of the physical map and nucleotide sequence of the cDNA revealed that it is composed of *bcr* and *abl* sequences^{18,19-21}. Analysis of genomic clones (Fig. 1C) showed that in λ 7 *bcr* first exon is linked to *abl* exon II, presumably by a splicing mechanism. The *abl* sequence in both λ 7 and λ 8 varies from the sequences of other *abl* cDNA molecules^{6,18} at nucleotide 1,355 (G changed to C), and this results in substitution of the amino acid Glu for Gln. The *bcr* sequence of λ 7 differs from the published corresponding sequence¹⁹⁻²¹ as well as from λ 8 (not shown) at position 194 (C changed to T). This results in a termination codon and the shortening of the open-reading frame by 55 amino acids. Experiments of RNase protection should determine if this mutation is common or a rare case. The identification of this new *bcr-abl* RNA junction in the ALL-1 cell line, in which the central region of the *bcr* gene is not rearranged and which lacks the 8.6 kilobase (kb)-CML-specific *bcr-abl* RNA (ref. 13), suggests that in the Ph¹-chromosome of ALL-1 the breakpoint is at the 5' region of *bcr*, probably within the first intron. The fused RNA will contain about 7 kb and therefore should comigrate in electrophoresis with the 7 kb normal RNA species of *abl* and *bcr* (ref. 6).

It was possible that the new transcript in ALL-1 encoded the 190K *abl* protein kinase detected in this cell line (C.M.C., unpublished). We therefore prepared antibodies specific to different regions of *bcr* and *abl* polypeptides. This was done by inserting fragments of cDNAs into a trpE expression vector²² and production of trpE fusion polypeptides in *Escherichia coli*. Polypeptides HHA, 1125, and H12 are encoded by the first *bcr* exon, the first several *bcr* exons, and *bcr* exons immediately upstream to *bcr-abl* junction point in the 8.6 kb mRNA respectively (Fig. 2C). Polypeptide 140 is encoded by the C-terminus

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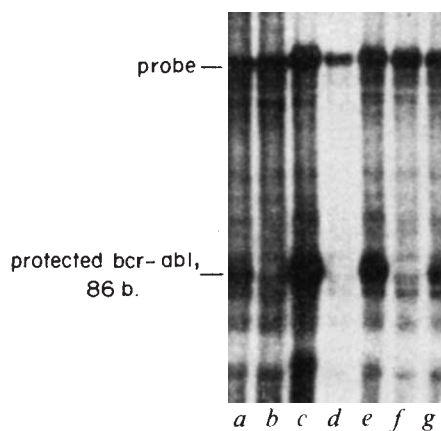


Fig. 3 Protection of *bcr-abl* RNA junction by RNAs from Ph¹-positive ALLs. RNAs were extracted from the leukaemic cells of the ALL patient O.R. (lane *a*), HL-60 cells (lane *b*), ALL-1 cells (lane *c*), Molt-4 cells (lane *d*), the cell line SUPB13 derived from an ALL patient (lane *e*), U-937 cells (lane *f*) and a mouse-human hybrid cell line containing the 22q⁻ of a Ph¹-positive ALL patient¹³ (lane *g*).

Methods. The 86 bp *Bgl*III-*Eco*RI junction fragment was isolated from λ 7 and cloned in reverse orientation into the Gemini-2 vector. The plasmid was linearized with *Hind*III enzyme and a 135 base RNA transcript was synthesized *in vitro* using SP6 enzyme and ³²P-CTP at specific activity of 400 Ci mmol⁻¹. Polyadenylated (2–3 μ g) RNAs were hybridized to 10⁶ c.p.m. of the probe and assayed as described elsewhere¹⁸.

Thus, unlike the v-abl protein (coded by the genome of Abelson murine leukaemia virus), which fractionates equally into the soluble and membrane fractions²³, the 190K protein is predominantly cytoplasmic.

The availability of the new *bcr-abl* cDNA made it possible to determine whether homologous transcripts are present in other Ph¹-positive ALLs. An 86-base pair (bp) *Bgl*III-*Eco*RI fragment containing the *bcr-abl* linkage of λ 7 was inserted in the reverse orientation into the Gemini 2 transcription vector. Radiolabelled anti-mRNA was synthesized *in vitro*, hybridized to cellular RNA, treated with RNase and analysed by electrophoresis. RNA from three Ph¹-negative human cell lines (Fig. 3, lanes *b*, *d*, *f*) did not protect the junction fragment. In contrast, RNAs from the leukaemic cells of a Ph¹-positive ALL patient (Fig. 3, lane *a*) and from three cell lines derived from similar patients (lanes *c*, *e* and *g*) protected the probe and therefore contained the "ALL-type" *bcr-abl* RNA transcript. The DNAs from these four patients were tested for rearrangement of the 5.8 kb central *bcr* region³ and were found to be negative (not shown). We conclude that expression of the new *bcr-abl* transcript is a general feature of Ph¹-positive ALLs. In these patients the chromosome 22 breakpoint(s) is slightly upstream to the position of the breakpoints in CMLs, and is likely to be within the first intron of *bcr*.

The results described here indicate that in Philadelphia-chromosome positive ALL, as in CML, the main consequence of the translocation is the synthesis of a *bcr-abl* mRNA and protein. The structural difference between the CML-specific 210K protein and the ALL-specific 190K protein is apparently reflected in the biological properties of the two proteins. The first protein probably triggers the chronic phase of CML, whereas the 190K protein is associated with the more aggressively malignant ALL. The *bcr-abl* fused gene in CML has the potential to be transcribed into an "ALL-type" *bcr-abl* mRNA through secondary rearrangements within the *bcr* gene or by a mechanism of alternative splicing. It is not impossible that the synthesis of an "ALL-type" mRNA will be associated with transition from the chronic to the acute phase of CML.

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Characterization and molecular cloning of a bovine lentivirus related to human immunodeficiency virus

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An infectious virus which causes persistent lymphocytosis, lymphadenopathy, lesions in the central nervous system (CNS), progressive weakness and emaciation was previously isolated from the leukocytes of cattle¹. Our present studies show that this virus encodes a reverse transcriptase (RT) with Mg²⁺ cation preference, replicates and induces syncytia in a variety of embryonic bovine tissues *in vitro*, and has a morphology most similar to the human immunodeficiency virus (HIV). Moreover, serologic analyses have demonstrated a conservation of epitopes between the major core protein of this bovine retrovirus and HIV. Shared antigenic determinants were also observed with other pathogenic retroviruses of the lentivirus subfamily. To resolve the phylogenetic relationship of this virus, proviral molecular clones were derived and used to determine the nucleotide sequence of the highly conserved RT domain. The sequence data and serologic analyses together show that this bovine retrovirus is a novel lentivirus related to HIV and other lentiviruses. We propose that this virus be tentatively named bovine immunodeficiency-like virus (BIV) to reflect its genetic relationship and biological similarity to HIV.

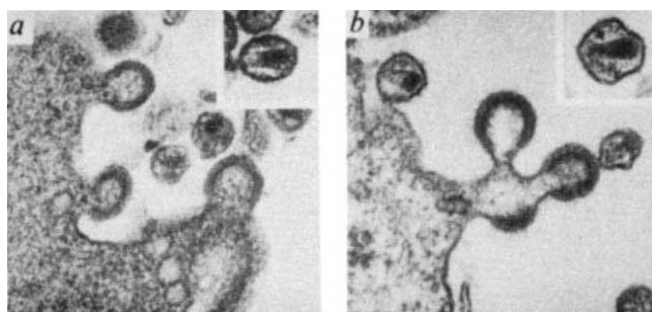


Fig. 1 Transmission EM of thin sections of BIV-infected bovine lung cells (*a*) and HIV-infected H9 lymphocytes (*b*) showing budding particles and typical mature BIV and HIV particles (inserts). Virus-infected cells were prepared for EM analysis as previously described⁵. All micrographs are $\times 48,000$.

The formation of syncytia in cell culture, morphology of the virus particle, and complexities of the disease syndrome caused by BIV were all suggestive of a lentivirus agent¹. To propagate and further characterize BIV, a series of primary bovine cell cultures were independently derived from embryonic tissues of a first-trimester male bovine fetus. Cells from all explanted tissues tested were infectible, including lung, thymus, testes, spleen, kidney, synovial membranes, choroid plexus, and brain. Virus production was assessed by syncytia formation, electron microscopy (EM), RT assay, and viral-specific antigen production as determined by indirect immunofluorescence assays (IFA) using antisera from cattle naturally or experimentally infected with BIV. The syncytia-inducing capability of BIV is similar to that of HIV and other lentiviruses^{2,3} and can be transferred to uninfected cell cultures by filtered tissue culture fluids or, more efficiently, by addition of cells from BIV-infected cultures. Approximately 24 hours after initial signs of cytopathic effects

in infected cultures, there is a rise in virus production as measured by EM, RT activity, and IFA. The BIV RT shows optimal activity using a poly(rA)-oligo(dT₁₂₋₁₈) template-primer and has a significant preference for Mg²⁺ as the cofactor, similar to HIV (ref. 4). Transmission EM of cells harvested from BIV-infected cultures reveal budding and extracellular virus with a lentivirus morphology identical to that described for HIV (Fig. 1*a* and *b*)⁵. The most striking feature is the presence of a condensed, variably electron-dense, bar-shaped core for both BIV and HIV mature extracellular particles which are 110–130 nm in diameter (Fig. 1*a* and *b* insets).

Polyclonal antisera were used to assess the serologic relationship between BIV and other lentiviruses. First, IFAs were used to test for gross cross-reactivity between BIV and HIV (Fig. 2*a* and *b*). With low dilutions of BIV antiserum, strong fluorescence was detected in the cytoplasm of HIV-infected lymphocytes (Fig. 2*a*) which was comparable in fluorescence intensity and distribution to HIV antiserum at threefold greater dilution (Fig. 2*b*). In contrast, HIV antiserum was only weakly reactive with BIV-infected cells (data not shown). Western blotting studies using these cross-reactive sera confirmed the IFA data and localized the recognized antigenic determinants to the major gag (core) proteins, p24 and p26 of HIV and BIV, respectively (Fig. 2*c*). Sensitive homologous and heterologous competitive radioimmunoassays (RIA) were also employed with a broader sampling of retroviruses. In the homologous RIA, HIV antiserum at limiting dilutions was used to precipitate ¹²⁵I-labelled HIV p24 core antigen (Fig. 3*a*). STLV-III, a primate lentivirus from macaques which shares immunologic and nucleotide cross-reactivity with HIV (refs 6–9), gives partial competition in this assay. BIV, other lentiviruses and various oncoviruses do not compete at all. In the heterologous assay, using BIV antiserum and ¹²⁵I-labelled HIV p24 (Fig. 3*b*), BIV, HIV, EIAV and STLV-III appear to give complete competition, although there are qualitative differences in the slopes of the curves. No other

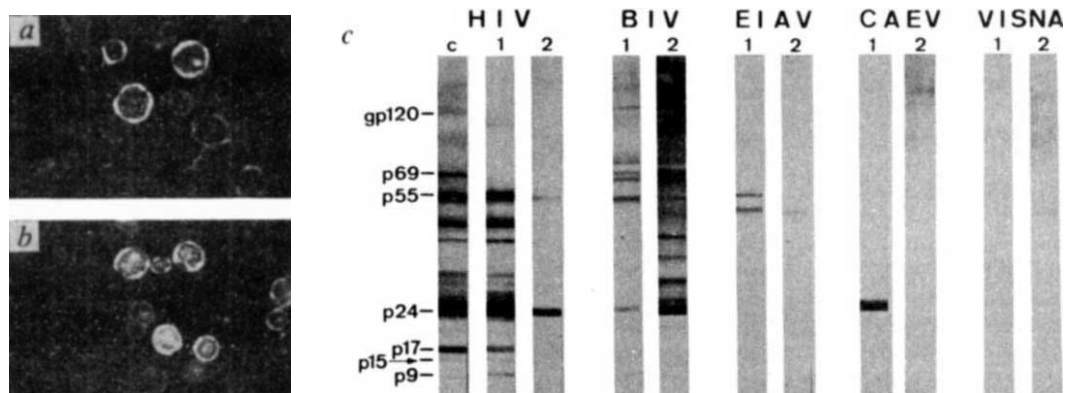


Fig. 2 Serologic analyses of cross-reactivity of polyclonal antisera to BIV and HIV by IFA and Western blot. *a*, HIV-infected H9 cells reacted with rabbit anti-BIV at 1:8 dilution for IFA. About 20% of the cells had bright cytoplasmic fluorescence, 50% showed moderate to weak fluorescence, and the remaining 30% were negative. Magnification $\times 275$. *b*, HIV-infected H9 cells reacted with rabbit anti-HIV at 1:24 dilution for IFA; the intensity and distribution of reactivity was similar to that seen with anti-BIV serum in *a* above. Magnification $\times 275$. H9 cells were negative in IFA using the rabbit anti-BIV or anti-HIV serum. *c*, Western blot analysis of rabbit anti-BIV and anti-HIV serum. Viruses tested were HIV, BIV, EIAV, CAEV and visna virus. Lane *c* is serum from an AIDS patient included as a positive control which shows reactivity to the major processed gag proteins, p24, p17, p15, p9, the unprocessed gag precursor, p55, the major outer envelope protein, gp120 and a pol product, p69 of HIV. Lanes 1 and 2, with each virus tested, were the rabbit anti-HIV and anti-BIV ser, respectively. Rabbit anti-HIV serum shows reactivity to the major core protein of HIV, p24 and several larger and smaller molecular mass bands which correspond to precursor and processed gag proteins of HIV also detected with AIDS serum. The rabbit anti-HIV serum shows cross-reactivity with BIV p26 and, in addition, CAEV p27 core protein. The rabbit anti-BIV serum shows reactivity to BIV p26 and HIV p24 and p55. The viral relationship of other faster and slower migrating bands in the BIV lane detected by the rabbit anti-HIV and anti-BIV sera are presently unresolved and under study. Prebleeds of the rabbits inoculated with HIV and BIV were uniformly negative against all viruses tested in Western blots.

Methods. Antiserum to BIV or HIV proteins was raised in rabbits by injection of detergent-disrupted sucrose gradient-purified whole virus. For IFA, BIV and HIV antisera were reacted with methanol-fixed HIV-infected H9 lymphocytes attached to glass slides. The secondary antibody for IFA was a fluorescein conjugated, affinity-purified goat anti-rabbit IgG (heavy and light chains) used at $20 \mu\text{g ml}^{-1}$. For Western blots, concentrated sucrose gradient-purified virus was detergent disrupted, electrophoresed in polyacrylamide gels, and transferred to nitrocellulose. Each lane contained $20 \mu\text{g}$ of disrupted virus protein. To limit nonspecific binding, each blot was pre-incubated with a 5% non-fat dry milk solution. Antisera were diluted 1:100 in buffer containing 1% gelatin. Bound antibody was detected with protein A conjugated to peroxidase and visualized with 4-chloro-1-naphthol. We used HIV proteins detected by the AIDS serum as molecular size markers.

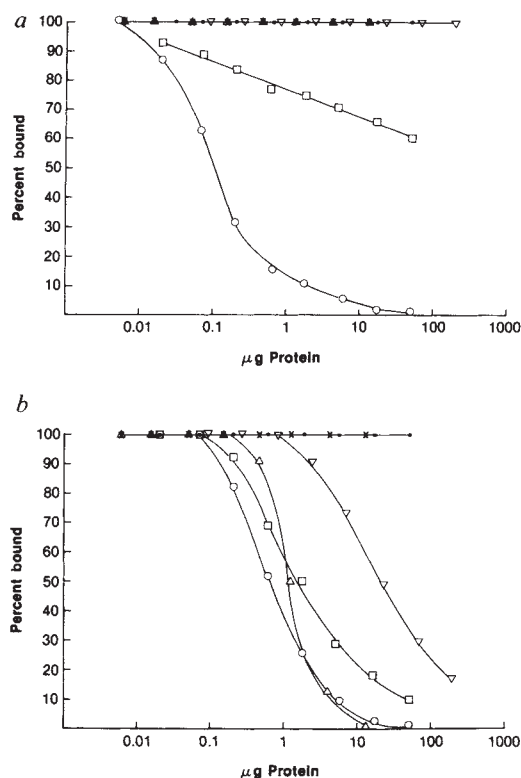


Fig. 3 Immunological relatedness of major core proteins of retroviruses using a competitive radioimmunoassay for the major gag (p24) protein of HIV. The viruses (or mock virus preparations) used to compete were HIV (○), BIV (△), HTLV-III (□), visna virus and CAEV (×), EIAV (▽) and FeLV, BLV, HTLV-I, HTLV-II, and uninfected concentrated H9 cell supernates (●). *a*, Homologous assay using ^{125}I -labelled HIV p24 and anti-HIV serum described in Fig. 2. *b*, Heterologous assay using ^{125}I -labelled HIV p24 and the anti-BIV serum described in Fig. 2.

Methods. Sucrose gradient-purified viruses were disrupted in detergent and tested for their ability to compete for the binding of ^{125}I -labelled HIV p24 at limiting dilutions of antiserum to BIV or HIV according to methods previously described⁹.

lentivirus or oncovirus gave appreciable competition. Thus, this assay indicates that the core antigens of the competing lentiviruses share some common antigenic determinants that can be detected with precipitating antibodies in the anti-BIV serum by RIA.

We^{5,10} and others^{11,12} have previously demonstrated an ancestral relationship between HIV and lentiviruses. The shared morphology and antigenic cross-reactivity between BIV and other lentiviruses, particularly HIV, provides strong evidence for a close evolutionary relationship for BIV and this group of retroviruses. To help quantify this genetic relationship, we derived a series of overlapping molecular clones of BIV proviruses from a recombinant lambda (λ) library made from genomic DNA of BIV-infected bovine cells (M.J.B. *et al.*, manuscript in preparation). These clones were selected for their ability to hybridize both to a complementary DNA (cDNA) made to BIV-specific viral RNA under high stringency and *pol* probes made from either visna virus or HIV under conditions of low stringency hybridization as previously described^{5,10}. The greatest amount of nucleic acid sequence homology between retroviruses exists in the RT domain at the 5' end of the *pol* gene; it is within this conserved region that systematic relationships for retroviruses have been derived¹⁰⁻¹⁴. We therefore concentrated our further efforts on the *pol* hybridizing regions of one of these clones (λ BIV 56). The nucleotide sequence homology between BIV and other lentiviruses over this region is 60% (300 base pairs, no gaps) and 52% of the amino-acid residues are identical (Fig. 4a). Inspection of this alignment indicates that BIV clusters

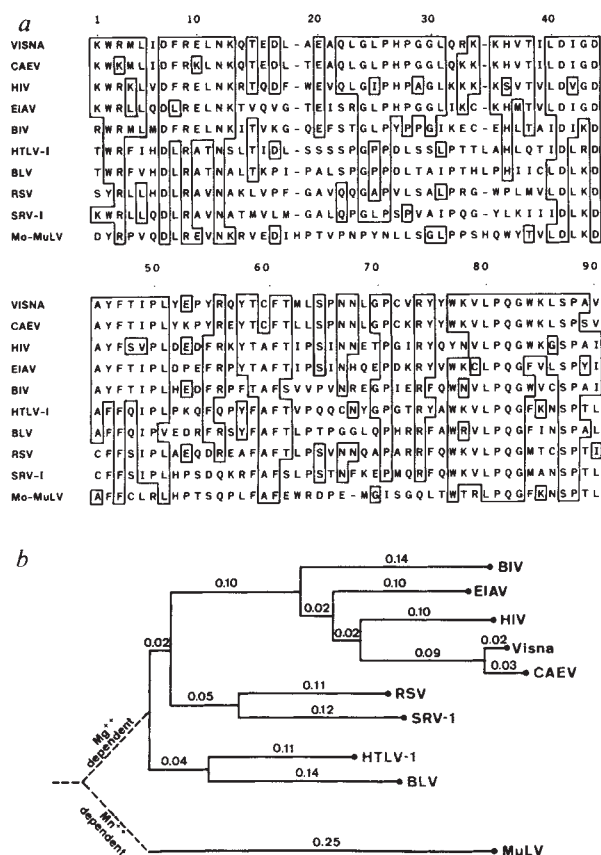


Fig. 4 *a*, Alignment of lentivirus *pol* genes with those of other retroviruses²⁴. The BIV amino-acid sequence was that predicted from the nucleotide sequence of the *pol* gene of λ BIV 56 (M.J.B. *et al.*, manuscript in preparation) derived by standard sequencing protocols^{25,26}. Those for CAEV¹¹, HIV²⁷, EIAV¹¹, visna virus¹⁰, HTLV-I²⁸, BLV²⁹, RSV²⁸, SRV-1¹³ and MuLV²⁸ were from published sequences. The alignment shown for each virus is generally that optimal for visna virus; slight improvements in other pairwise alignment scores can be made by minor shifts in the placement of gaps. Boxes are drawn around identical residues when at least three lentiviruses share that residue. *b*, Fitch-Margoliash phylogenetic tree¹⁵ of retroviral relationship based on the *pol* gene region shown in *a*. Branch lengths are in units of $-\log M$, where M is the frequency of matching residues. The tree was rooted with the type C oncovirus, MuLV (Moloney), as the outgroup. The average percent standard deviation of the tree was 4.8.

with the lentiviruses. Moreover, from comparison of the amino-acid sequence alignment of BLV and BIV (Fig. 4a), our BIV clone is not closely related to BLV (37% shared amino acids). A Fitch-Margoliash phylogenetic tree¹⁵ derived for these retroviral *pol* gene segments (Fig. 4b) was rooted with murine leukaemia virus (MuLV) as the outgroup taxon because it consistently had the lowest alignment scores with other retroviruses and because the RT of MuLV preferentially uses Mn^{2+} as a cofactor, whereas the rest use Mg^{2+} . Three major subgroups are evident among those viruses whose RT prefer Mg^{2+} as cofactor (Fig. 4b). These are the lentiviruses (including BIV), the RSV/SRV-1 group (type D and avian type C viruses) and the HTLV-1/BLV group. We also used the PAUP program (D. L. Swofford, Illinois Natural History Survey, Champaign, Illinois) and the PROTPARS program from the PHYLIP package (J. Felsenstein, University of Washington, Seattle, Washington) to derive maximum parsimony trees (not shown) which also identified these three major subgroups. Within the lentiviruses, CAEV and visna virus are clearly most closely related with 90% shared amino acids. While the topology of the Fitch-Margoliash tree (Fig. 4b) has HIV positioned slightly closer to the visna

virus/CAEV pair than either BIV or EIAV, the branching order of these taxa was variable in the maximum parsimony trees. Therefore, we consider the true branching order of the lentiviruses unresolved and conclude that HIV, BIV, EIAV and the visna virus/CAEV pair are about equidistant from each other.

Our present studies describe a new and distinct member of the lentivirus subfamily of retroviruses isolated from domestic cattle which shares structural, serologic, and genetic features in common with HIV. The *in vitro* and initial *in vivo* response to BIV infection also bears some resemblance to HIV. Experimental infection of calves with BIV results in a mild persistent lymphocytosis and a prominent lymphadenopathy early (within 3–12 weeks post-inoculation) in the infection which is similar to the persistent generalized lymphadenopathy considered to be part of the AIDS-related complex, or ARC, which is seen in the same population groups at risk of developing classical AIDS^{16,17}. It is not clear whether the increase in lymphocytes is due to helper or suppressor cells, or both. Whether BIV causes immunosuppression leading to secondary infections and/or deaths has not yet been investigated since suspect unhealthy individuals are normally culled from herds and killed. In one animal naturally infected with BIV, a mild lymphocytic perivascular cuffing in the brain was observed, although the precise involvement of BIV in CNS lesions was not determined¹. Whether BIV infects lymphocytes and/or monocytes like HIV and other lentiviruses² and whether the lymphocytosis is a direct result of cells being infected with BIV or is a cellular response to ward off the infection is also not known. But infected cattle do respond with humoral antibody.

The isolation of two pathogenic retroviruses from cattle, BLV and BIV, related respectively to the only known human retroviruses, HTLV-I (–II) and HIV, raises several important questions about BIV relevant to both veterinary and human medicine. First, how prevalent and severe is BIV infection in cattle? Second, is BIV in cattle a problem that has gone undiagnosed or misdiagnosed due to the overriding presence of other viruses, like BLV which also causes lymphocytosis? We speculate that this may be the case and in some instances BLV may occur as a secondary infection in BIV-infected animals, much like HTLV-I does in AIDS patients^{18–20}. Studies are in progress to determine the serologic status of selected herds which will provide information to help resolve these questions. Lastly, are there any potential risks for man in handling BIV-infected animals and products? At present, there is no direct evidence to support this notion. Man has maintained close associations with several domesticated animals, including sheep, goats and horses, which are known to carry lentiviruses. More recently, a lentivirus agent appears to have been isolated from household cats²¹. Yet, there is no evidence for an animal-to-human transmission for any of these viruses. It should be noted that a STLV-III-like virus (HIV-2), has been detected in and isolated from humans; however, its pathogenesis in man remains unresolved and evidence for a monkey-to-human transmission is still speculative^{22,23}. Therefore, in the absence of empirical data for BIV, serologic analyses will be conducted to determine the antibody status of individuals considered to be at risk from BIV infection.

The present studies have discovered the presence of cross-reactive epitopes between core proteins of BIV and HIV. This indicates that sensitive type-specific assays will be needed to discriminate between BIV and HIV proteins. In this regard, the molecular clones of BIV described here may be of use in targeting and genetically engineering highly specific antigens for these studies. Moreover, the abundant availability of cattle in comparison to primates, the antigenic and genetic relationship as well as the biological similarities to HIV and the rapid sequence of events in disease induction suggests that BIV is a relevant lentivirus candidate for the study of viral interventional strategies applicable to AIDS. These studies may be of benefit to animals as well as man.

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Characterization of cellular proteins recognizing the HIV enhancer using a microscale DNA-affinity precipitation assay

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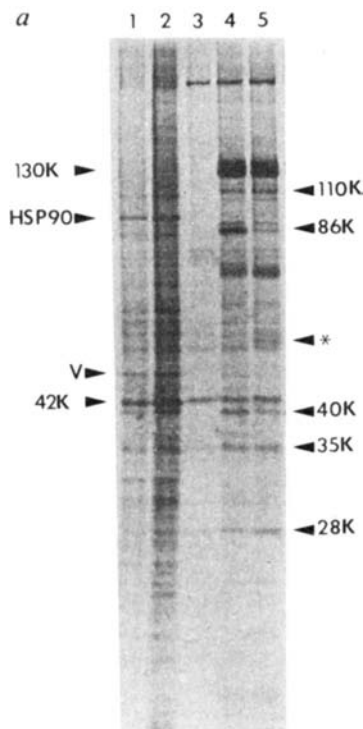
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Numerous nucleic acid sequence motifs have been identified as transcriptional regulatory elements, and proteins involved in the control of gene replication and transcription have been successfully purified using nucleic acid affinity procedures^{1–5}. We have developed an assay that allows direct characterization of cellular proteins binding to the enhancer element of the human immunodeficiency virus (HIV), which is analogous to immunoprecipitation techniques. In extracts of H9 cells, a human CD4⁺ lymphoblast line clonally selected for its ability to support HIV replication^{6,7}, we find a class of proteins that interact specifically with the HIV enhancer. Reproducible high-resolution, two-dimensional gel electrophoresis has allowed us to resolve these proteins into two major sets. One set is common to H9 cells, two human B-lymphoblast lines, and a phytohemagglutinin (PHA)-stimulated human CD4⁺ lymphoblast line (Jurkat). A second set of proteins is found only in H9 cells. Thus, with this assay we have identified HIV enhancer-binding proteins that are constitutive or inducible and that are cell-type specific.

The principle of our assay is to separate specific DNA-binding proteins from complex mixtures of proteins, then resolve them by gel electrophoresis. Synthetic oligonucleotides or restriction fragments are covalently modified by the addition of a biotin group, mixed with either whole-cell or nuclear-protein extracts, and the protein-nucleic acid complexes are collected on streptavidin-agarose beads. The proteins are eluted from the beads and resolved by standard gel electrophoresis techniques. A routine assay can be accomplished in 3 h, excluding gel running time. The assay can thus be used to characterize directly proteins from individual cell lines that recognize a particular nucleic acid sequence and to compare such proteins to those that bind to other regulatory elements. Also the assay can be used to demonstrate cell-specific or response-specific binding proteins by analysing different cell lines under various growth conditions. The assay can also reveal those proteins that interact with a nucleic acid-protein complex, in addition to those that bind specifically to the nucleic acid. This simple assay is meant to complement current, widely-used procedures such as gel retardation and nuclease protection assays of protein-nucleic acid interactions.

Deletion analyses of the HIV long terminal repeat (LTR) have revealed an enhancer element between -105 and -80 base-pairs (bp) upstream of the transcription start site⁸⁻¹⁰ and both positive¹⁰ and negative^{8,10} control regions at sites upstream of this enhancer element. Here we describe interactions of cellular proteins with the genetically well-characterized HIV enhancer. This enhancer (-105 to -80) contains a 10-bp direct repeat, GGGACTTTC, that is 100% homologous to enhancers of two DNA viruses, SV40 (ref. 11) and human cytomegalovirus¹², and to a sequence in the κ -immunoglobulin light-chain gene enhancer region¹³. As probes, we synthesized oligonucleotides containing two complete, tandem copies of the region extending from -106 to -79 (Fig. 1) which we refer to as HIVEN3c 1 and 2. Parallel mutant oligonucleotides called HIVEN3m 3 and 4 contain three point mutations within each decamer repeat. The mutant construct was chosen because it had previously been shown to abrogate PHA plus 12 *O*-tetradecanoyl phorbol 13-acetate (TPA)-inducible activity of HIV-LTR reporter gene constructs¹³. Figure 2a, lane 4, shows the pattern of proteins that bind to the wild-type annealed oligonucleotides HIVEN3c1/2 using nuclear extracts prepared from H9 cells. The pattern is complex as proteins of relative molecular mass (M_r) 130K, 110K, 86K, 40K, 35K and 28K are easily detected. These proteins are not detected in control reactions lacking biotinylated oligonucleotides (see Fig. 2a, lane 3). Using mutant probe HIVEN3m3/4 there is a significant decrease in binding of the protein(s) migrating at 86K (Fig. 2a, lane 5) whereas the remaining proteins are still precipitated. Comparison of the protein patterns with these two DNA fragments define four classes of proteins: (i) nonspecific proteins such as actin (42K) that are precipitated by the streptavidin-agarose beads; (ii) proteins



enriched by binding the wild-type fragment and the mutant fragment, such as 130K and 110K; (iii) a protein(s) preferentially associated with the wild-type fragment, 86K; and (iv) a protein(s) (designated by an asterisk in Fig. 2a) that bind(s) to the mutant oligonucleotide probe.

To characterize the HIV enhancer binding proteins better, we have used a high-resolution, two-dimensional (HR2D) gel electrophoresis system. Factors that contribute to the reproducibility and high resolution of the gels have been previously described¹⁹. All HR2D gels presented in this report are oriented as follows: the more acidic proteins on the left side of each panel, and apparent decrease in molecular size oriented top to bottom. Four regions of different HR2D gels are presented in Fig. 2b: A is the H9 whole cell pattern; B shows proteins precipitated in a reaction without biotinylated oligonucleotide (control); C, proteins precipitated complexed with the biotinylated HIVEN3c1/2; D proteins precipitated complexed with the biotinylated HIVEN3m3/4. The M_r range in each panel is between 120K and 50K. As can be seen in C, spots specifically enriched by HIVEN3c1/2 are designated A (HIVEN86A), B

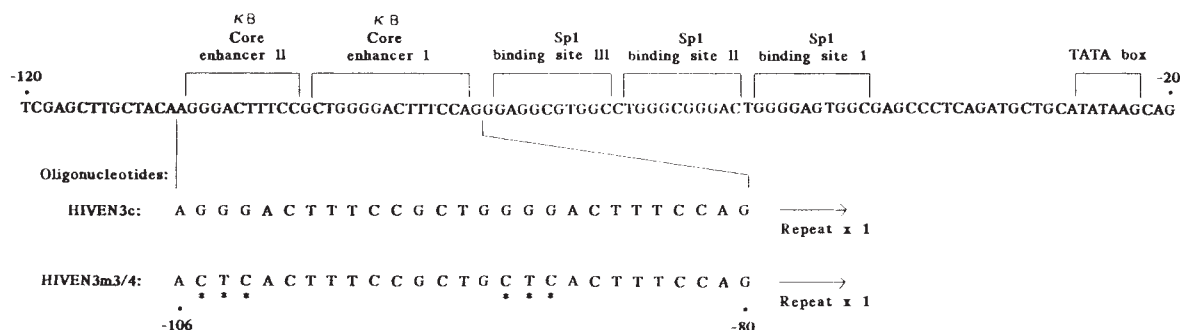


Fig. 1 The HIV-LTR sequences and oligonucleotide constructs used here. The alterations in the enhancer mutant are marked by an asterisk over the substituted bases. Oligonucleotides were synthesized on an Applied Biosystems synthesizer, Model 380A, on a 1 μ M scale, and then purified by polyacrylamide-urea gel electrophoresis¹⁴. The Sp1 sites designated here were reported initially in ref. 15. The core enhancer or 'kappa B (κ B)' elements are described in refs 9 and 13, and coincide with our unpublished studies.

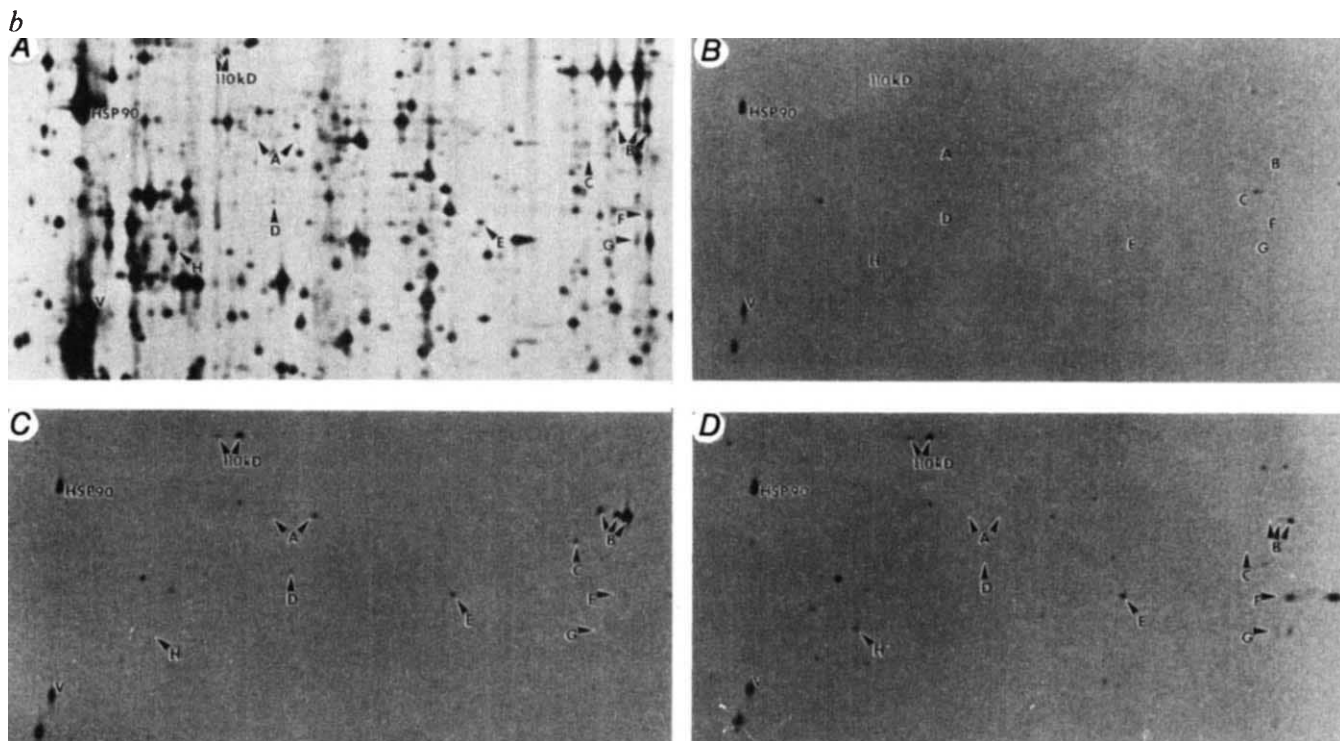
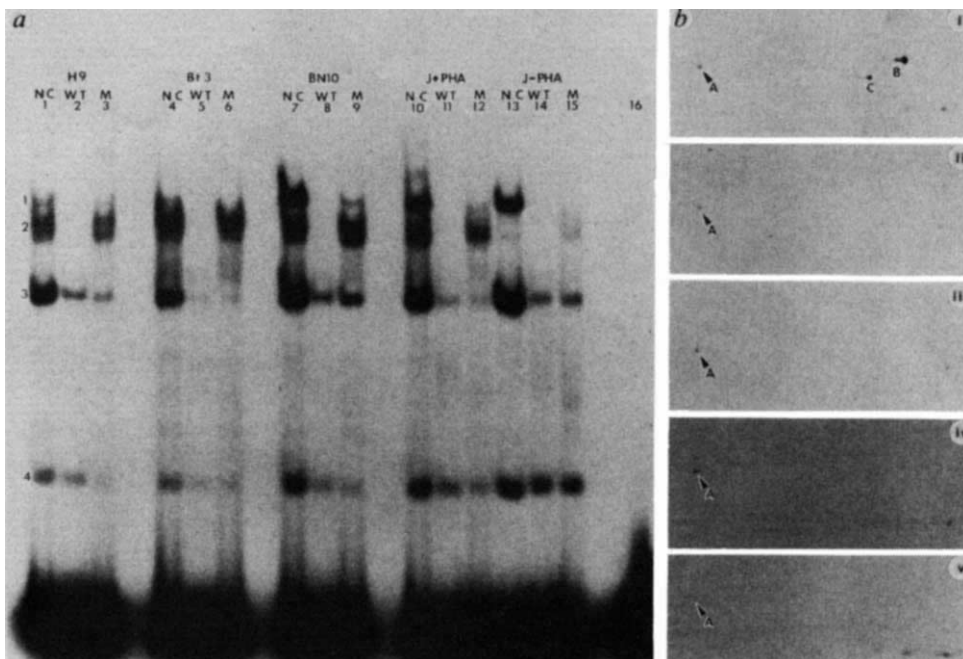


Fig. 2 *a*, Alteration of bases -105 to -103 and -90 to -88 in the HIV enhancer is sufficient to disrupt 86K binding. In this assay 6×10^8 cells were labelled in 1.0 ml DMEM-met containing 1.5 mCi 35 S-methionine at 37 °C for 30 min in a 35-mm dish. Lanes 1 and 2 have 1/1,000th and 1/200th of the crude nuclear extract used for each reaction respectively. Lane 3 shows the proteins that are recovered when all the ingredients of the reaction except biotinylated probe are added; lane 4, the proteins recovered with the biotinylated HIVEN3c1/2 oligonucleotide, lane 5, proteins recovered with the biotinylated HIVEN3m3/4 oligonucleotide. Exposure was for two days on XAR film. *b*, Two-dimensional gel analysis of HIV enhancer-binding proteins.

Methods. *a*, Photoprobebiotin was from Vector Lab, streptavidin-agarose from Bethesda Research Labs, all radioactive reagents from Amersham. The sun lamp used in the nucleic acid biotinylation reaction was GE Type RSM, 275 W. All reagents used are of the highest quality available. Cells used were human T-lymphoblast line, H9, Jurkat cell line (both are CD4⁺ surface-receptor positive) and two B-lymphoblast cell lines SK-CML8-Bt3 and SK-CML8-BN10. The following solutions were used: Solution A, 30 mM TrisCl, pH 8.0, 10 mM KCl, 0.01% Na₂S₂O₃; solution B, 50 mM TrisCl, pH 8.0, 0.01% NP-40, 20% glycerol, 1.5 mM MgCl₂, 0.01% bromophenol blue (stored at -20 °C as aliquots); solution C, 1 mg ml⁻¹ DNase 1, 500 mM TrisCl, pH 7.0, 50 mM MgCl₂ (stored at -20 °C as aliquots); solution D, 100 mM TrisCl, pH 7.8, 1 M NaCl, 10 mM EDTA (stored at -20 °C as aliquots). Preparation of biotinylated oligonucleotides: Typically 2 nmol of single-strand oligonucleotide were biotinylated in water, and annealed to its complementary strand. Photoprobebiotin (1 µg µl⁻¹, ref. 16) was added to the nucleic acid solution in an amount equal to the mass of nucleic acid. The tube was placed in an ice water bath so that the surface of the mixture was 7 cm below the sun lamp, a glass microscope slide was placed over the mouth of the tube, and the mixture exposed to the sun lamp for 15 min. The biotinylated strand was annealed to its complementary strand by adding the complementary strand and 1/10th vol. solution F. The mixture was placed at 80 °C for 2 min and then allowed to cool at room temperature. The mixture was adjusted to pH > 8.5 by addition of 1 M TrisCl, pH 9.0, and extracted three times with equal volumes of *n*-butanol. The volume was adjusted to 10 pmol µl⁻¹ nucleic acid, and stored at -20 °C. Other uses of biotin-avidin schemes for preparative affinity chromatography were reported during the development of this assay²⁻⁵. Preparation of nuclear extracts: A typical assay contained the proteins extracted from the nuclei of 1×10^8 cells (lymphoblasts used in this study are maintained at 5×10^5 – 1×10^6 cells per ml in RPMI 1640 + 15% FCS). All labellings were done in DMEM-met as described in figure legends. After labelling, the cells were pelleted by a 30 s spin at 500g, washed two times with PBS (pH 7.3), washed one time in solution A, and then suspended in 1.0 ml solution A. These procedures were developed from modifications of those in refs 1 and 17. The cells were disrupted by 10–20 strokes in a 1.0 ml dounce homogenizer (type B). The crude nuclei were pelleted by a 15-s spin at 1,000g and suspended in solution B. A final extraction volume was 1.4 ml containing 150 µl of nuclei, plus 914 µl of solution B, 140 µl of solution C, and 196 µl of 3 M KCl (420 mM KCl final concentration) per 3×10^8 harvested cells. The extraction occurred at 4 °C on a slowly rotating wheel for 30 min. The extract was then spun for 5 min at 13,000g at 4 °C. The supernatant was removed and the pellet rinsed with 1.0 ml of solution B, spun and the supernatant combined with the original volume of extract. Solution B was added to adjust the KCl to a final concentration of 100 mM. The extract was preadsorbed (20 min, room temperature (RT)) with streptavidin-agarose, by adding 1.2 ml of extract to a 75 µl pellet of agarose beads. The mixture was spun at 13,000g (5 min, RT). Binding assay: competitor nucleic acid (poly-(dI-dC)-(dI-dC), Pharmacia) was added first and mixed with the extract (15 min, RT). Competitor was used at 40× mass of oligonucleotide probe. Oligonucleotide was 100 pmol per ml of mix unless otherwise noted. Mixture was then rotated (20 min, RT) and spun (2 min, 13,000g). Supernatant then mixed with 35 µl pellet of streptavidin-agarose (20 min, RT). The mix was spun for 15 s at 13,000g, the supernatant removed and beads washed three times with solution B containing 100 mM KCl, 5 mM NaF and 1.5 mM MgCl₂. Proteins extracted for one-dimensional gel analysis by addition of 50 µl of solution D, and 1 µl of solution E. One-dimensional gel analysis as previously described¹⁸. Immediately before loading the gel, the beads were boiled for 3 min and spun for 2 min at 13,000g. *b*, H9 cells (8×10^8) labelled for 30 min at 37 °C with 1.5 mCi 35 S-methionine, and then the cells rinsed twice with PBS, and extracted as described above for nuclei. Note the final KCl concentration was 300 mM. After 30 min at 4 °C the supernatant was separated from the cellular debris by centrifugation at 4 °C for 15 min at 13,000g. The supernatant was adjusted to a final concentration of 100 mM KCl by addition of buffer B, and processed as above. Extraction of protein from the streptavidin-agarose, biotinylated-oligonucleotide complex was accomplished by adding a solution of 50 mM TrisCl, pH 8.0, 3% SDS and 10% BME to the beads and boiling for 3 min. The beads were then spun from solution, the supernatant removed, and 200 µl H₂O added to the beads. The beads were again spun from solution and the supernatants combined, frozen, lyophilized, and prepared for two-dimensional gels by re-solubilization in a solution containing 9.95 M urea, 4.0% NP-40, 2% ampholytes (LKB, pH 6.8), and 100 mM DTT. Two-dimensional gel analysis as previously described¹⁹. All panels represent a region from two-dimensional gel fluorograms of the region between apparent *M*_s of 120K and 50K. The gels were pH 3.5–10, first dimension isoelectric focusing (acidic proteins on left), and 10% acrylamide second dimension gels. A, whole cell pattern, ~300,000 d.p.m. TCA-precipitable proteins loaded; B, proteins precipitated from reaction mixture containing everything except biotinylated oligonucleotide; C, proteins precipitated in the presence of 100 pmol HIVEN3c; and D, proteins precipitated in the presence of 100 pmol HIVEN3m3/4. Heat shock 90K, HSP90; vimentin V are noted as markers. A, gel exposed to XAR for 26 days. B–D gels exposed to XAR film for five days.

Fig. 3 *a*, Gel retardation assay using HIV enhancer probe. *b*, Affinity assay of whole cell lysates.

Methods. *a*, 12-bp double-stranded oligonucleotide derived from the HIV enhancer (5'-GGGACTTTCCAG-3') was cloned into the *Hinc*II site of pBSM13+ (Stratagene). To make the probe the cloned sequence was excised from the plasmid as a 63-bp *Hind*III-*Eco*RI fragment, labelled at both ends with T4 polynucleotide kinase, and gel purified. Assays were carried out as previously described²⁸. Reaction volumes were 20 μ l and contained 10 mM Tris, pH 7.5, 2 mM MgCl₂, 1 mM DTT, 1 mM EDTA, 5% (vol/vol) glycerol, 50 mM NaCl, 50 μ g ml⁻¹ poly-(dI-dC):poly-(dI-dC), 10 μ g ml⁻¹ pUC13 DNA. Each reaction contained 20,000 c.p.m. probe and 10 μ g whole-cell extract protein. In addition, lanes 2, 5, 8, 11 and 14 contained 10 pmol biotinylated HIVENc1/2 oligonucleotide competitor, and lanes 3, 6, 9, 12 and 15 contained 10 pmol biotinylated HIVENm3/4 competitor. Extracts were prepared, as described below, from H9 cells (lanes 1-3), SK-CML8-Bt3 (lanes 4-6), SK-CML8-BN10 (lanes 7-9), logarithmically growing Jurkat cells treated for 20 h with 1 μ g ml⁻¹ PHA (lanes 10-12), and untreated logarithmically growing Jurkat cells (lanes 13-15). Lane 16 contains the probe with no added extract. *b*, Cells (4×10^8) were labelled for 30 min at 37 °C with 1.5 mCi ³⁵S-methionine and rinsed twice with PBS, then extracted as above. Final concentrations in the extraction solution were 300 mM KCl and 50 mM NaFl. Two-dimensional gel sample preparation was as described in legend to Fig. 2b, and the same type of two-dimensional gel described in Fig. 2b was used to separate precipitated proteins. Panel i represents the HIVEN86A and B regions of H9 cells. The same region is presented for SK-CML8-Bt3 (panel ii), SK-CML8-BN10 (panel iii), Jurkat + PHA (panel iv), and Jurkat - PHA (panel v). A denotes the more basic, abundant form of HIVEN86A; B denotes the HIVEN86B polypeptides and C denotes the 83K polypeptide. Exposure was on XAR film for 12 days for H9, SK-CML8-Bt3 and SK-CML8-BN10; 5 days for the Jurkat $\pm$ PHA gels.



(HIVEN86B), C (83K) and D (78K). These spots are not observed in a reaction lacking biotinylated oligonucleotide (Fig. 2b, B). The two-dimensional gels reveal that the band corresponding to 86K (Fig. 2a) resolves into two clusters of proteins A and B, which may or may not be related. Also, spots C and D, like A, bound exclusively (at this level of exposure of the gels to film) to HIVEN3c1/2. E and F represent proteins recovered on both HIVEN3c1/2 and the mutant oligonucleotide HIVEN3m3/4 (Fig. 2b, compare C with D), with the mutant probe usually binding more of each. G and H, though recovered by both probes, are very abundant in the whole-cell pattern (Fig. 2b, A). The degree of enrichment of HIVEN86A and B polypeptides in the presence of the enhancer oligonucleotide is significant (Fig. 2b, compare C with A). The gel in panel A contained ~10-fold more protein and was exposed five times longer than the gel in panel C.

The analysis of H9 cellular proteins that interact with the HIV enhancer oligonucleotide was extended to a study of other lymphoblast cell lines. Different groups^{20,21} have described the activation of HIV replication and production of virus from latently-infected cells by agents that induce lymphokine synthesis and secretion. Jurkat cells have been used extensively in studies of the production of interleukins in response to various external stimuli. By treating Jurkat cells with a combination of PHA and TPA^{10,13}, or either agent individually¹⁰, transient expression of a reporter gene linked to the HIV-LTR was shown to be elevated compared to similarly transfected Jurkat cells grown in normal media. This response was amplified if the HIV trans-activator gene (*tat-III*) was co-transfected with the HIV-LTR reporter gene construct^{10,13}. Induced expression of the reporter gene was shown to coincide with the appearance in nuclear extracts of an activity that retards the migration of a DNA fragment containing the HIV enhancer sequence in a gel retardation assay¹³. This activity was proposed to be identical

to the NF- κ B factor that is detected in κ -immunoglobulin light chain-producing cell lines by the gel-retardation assay^{22,23}. We therefore examined induction of HIVEN3c-binding proteins in stimulated Jurkat cells, and the relationship of these proteins to those found in H9 cells. We included two human B-lymphoblast cell lines expressing exclusively either κ - or the λ -immunoglobulin light chains²⁴. The κ -immunoglobulin-producing cell line provides a putative NF- κ B-positive control for comparison with the PHA-induced Jurkat cells, and the λ -immunoglobulin producing cell line was chosen to determine if they possess similar and/or unique proteins.

To address this issue we carried out a series of gel-retardation assays²⁵⁻²⁷ using a cloned HIV enhancer 12-bp probe (-GGGACTTTCCAG-) in addition to the microscale affinity assay. Figure 3a shows that protein extracts from each of the cell lines assayed contained activities that bind to this sequence element. These patterns are more complex than previously reported¹³. The specificities of these interactions were determined by the addition to the retardation reactions of competitor oligonucleotides that were the same biotinylated probes used in the binding assays. Bands 1, 3 and 4 are variably detected in assays of each cellular extract, and are variably competed by both wild-type and mutant HIVEN3 oligonucleotides. But band 2 (a poorly resolved doublet) is found in H9 cells, in both κ - and λ -light chain producing B-lymphoblast lines, and is substantially induced in Jurkat cells upon stimulation with PHA using conditions shown to be sufficient to induce transcription of HIV-LTR linked reporter genes¹⁰. This behaviour correlates with the activity referred to as NF- κ B in that lectin-stimulated Jurkat cells and κ -immunoglobulin-producing B lymphoblasts both form the band 3 complex. The λ -immunoglobulin producing cells also appear to have an identical activity (Fig. 3a, BN10, band 3).

In the corresponding microscale affinity assays of similar

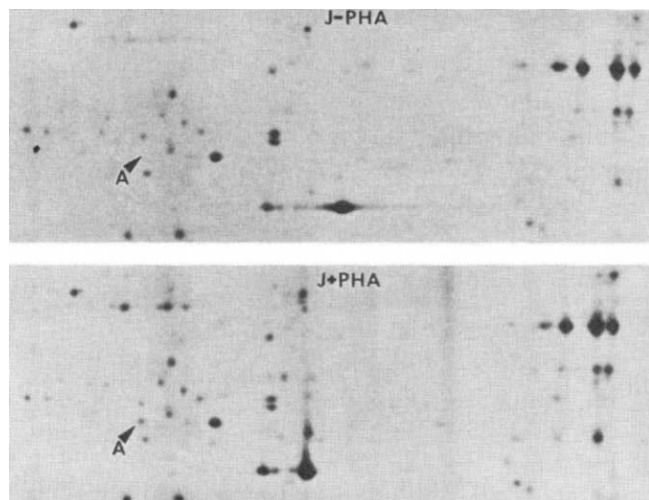


Fig. 4 Two-dimensional gel analysis of whole cell lysates from Jurkat cells $\pm$ PHA. Cells (3×10^8) were labelled for 30 min at 37 °C with 1.5 mCi ^{35}S -methionine. Cells treated with PHA ($1 \mu\text{g ml}^{-1}$) for 48 h before preparation of extract. All two-dimensional gel sample preparation and procedures as described in legend to Fig. 2b. Panel J – PHA shows the HIVEN86A region of the unstimulated Jurkat cells. Panel J + PHA shows the same region for the stimulated Jurkat cells. The letter A designates HIVEN86A. Exposure was for 26 days on XAR film.

extracts which have been metabolically labelled (Fig. 3b, i–v) it is evident that the HIVEN86A proteins are present in H9 cells and both B-lymphoblast cell lines (Fig. 3b, i–v). In Jurkat cells, stimulation with PHA results in a significant increase of this protein binding to the HIVEN3c1/2 (Fig. 3b, panel iv compared to v). Thus, the binding of the HIVEN86A proteins to the HIV enhancer fragment correlates with the appearance of band 3 in the gel-retardation assay. In contrast, the HIVEN86B proteins are detectable only in the H9 cellular extracts (Fig. 3b, panel i). Firm assignment of proteins to gel-shift bands awaits purification of each polypeptide. Nevertheless, the microscale affinity assay permits direct visualization of sequence-specific binding proteins whereas gel retardation analysis only allows indirect detection of a specific protein–nucleic acid interaction.

As demonstrated for H9 cells, the binding assay allows the identification of specific nucleic acid-binding proteins in HR2D gel patterns derived from separation of whole cell extracts. The PHA-induced HIVEN86A protein is also detected in the corresponding region of a HR2D gel separation of whole cell extracts of stimulated Jurkat cells (Fig. 4, J + PHA panel). At this level of exposure no HIVEN86A can be detected in the extracts from unstimulated cells (Fig. 4, J – PHA panel) consistent with the significant difference seen in both types of binding assay. Whether PHA induces the synthesis and/or post-translational modification of HIVEN86A is not yet evident. But the identification and resolution of the specific HIV enhancer-binding proteins on HR2D gels permits direct determination of the effect any specified manipulation of the cell has on the behaviour of each polypeptide. General application of this approach to the study of defined nucleic acid control sequences may provide substantial biological information with or without pure protein. This report represents the initial step in the systematic application of the binding assay in conjunction with HR2D gels to define inducible and cell type-specific cellular proteins that interact with nucleic acid motifs determined to be of regulatory significance. Database analysis (J. L. Garrels and B.R.F., submitted) provides the means to quantify and link all such observations into an accessible, comprehensive and quantifiable system for the study of protein effectors of gene regulation.

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Note added in proof: Since acceptance of this paper two reports describing PMA effects on genes linked to the HIV enhancer have been published that are consistent with previous studies^{29,30}.

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The *Drosophila* developmental gene *snail* encodes a protein with nucleic acid binding fingers

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Pattern formation in the *Drosophila* embryo requires the concerted expression of maternal and zygotic genes. At least nineteen genes, twelve of which are maternally expressed, are involved in the establishment of dorsal–ventral polarity^{1–4}. Mutations in any one of these genes result in distinct alterations of cell fates and in the formation of an abnormal dorsal–ventral pattern¹. Mutants of the ‘dorsal group’, eleven of the maternal genes, have a common recessive phenotype similar to that described for *dorsal*, in that cells located at ventral and lateral positions assume dorsal fates and ventral structures fail to develop. Thus the dorsal group gene products may be involved in the establishment of a gradient of positional information along the dorsal–ventral axis^{1,3}. We have cloned *snail* (*sna*), a zygotic gene, whose expression is essential for the correct specification of dorsal–ventral pattern. In this report, we present evidence that the complementary DNA-deduced protein product of *sna* contains five copies of a nucleic acid-binding finger motif previously identified in two transcription factors^{5,6}, and in the protein product of several putative regulatory genes^{7–11}.

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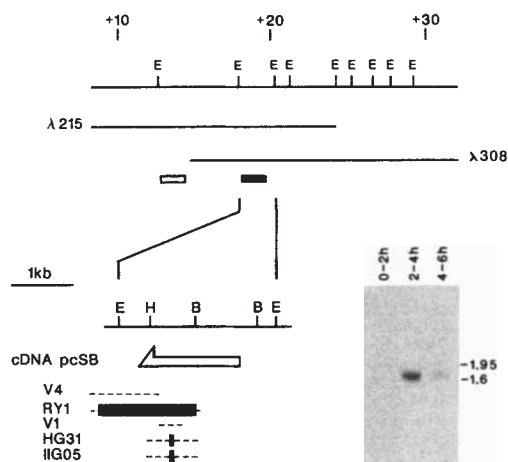


Fig. 1 The *sna* locus. The top lines represent the molecular map of the central region of the *sna* chromosomal walk with two representative recombinant phages, λ 205 and λ 308. *Eco*RI sites are shown. Coordinates in kb are positive in the proximal direction with respect to the chromosome 2 centromere. Box segments denote restriction fragments to which clones from an embryonic cDNA library hybridize. The solid box corresponds to the *sna* cDNA clone, pcSB. The open box is a still uncharacterized transcript. Below, the *sna* cDNA region is enlarged to show the position of the cDNA and of *sna* mutant breakpoints within this region. The region where the breakpoints are located is indicated by dashed lines. Filled boxes indicate the size of the deletions. *RY1*, the strongest allele, is X-ray induced. Two strong alleles *HG31* and *IIG05* were EMS-induced as were the two weak alleles *V1* and *V4* (ref. 4 and P. Simpson, unpublished results). Inset, Northern blot of embryonic poly(A)⁺ RNA (20 μ g per slot) probed with pcSB. The hours of development after egg-laying are indicated. Size markers are in kilobases.

Methods. Mutant breakpoints were mapped by comparing restriction fragment patterns of DNA from *sna* mutants (digested by *Eco*RI, *Bam*HI, *Hind*III, *Sal*I, *Xho*I) with that of DNA from wild-type stocks. Southern blots were probed with recombinants from the walk and the breakpoints subsequently confirmed by hybridization with pcSB. cDNAs were isolated from a 3–12 h embryonic cDNA library¹⁴. The direction of transcription was established by hybridizing Northern blots with single-strand probes from pcSB and pcSBI. Poly(A)⁺ RNA was prepared from staged Canton S embryos, denatured by formaldehyde and electrophoresed on 1% agarose-formaldehyde gels²⁵. Autoradiograph exposure was for 4h.

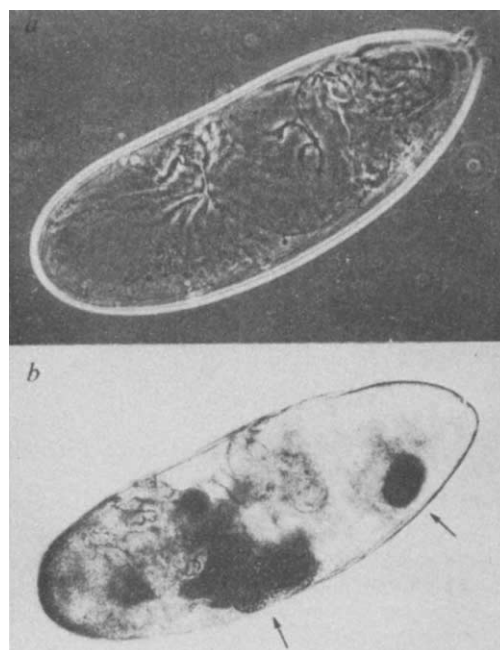


Fig. 2 *Sna* phenocopies induced by injection of anti-sense RNA. *a*, Homozygous *sna*^{IIG05} embryo. *b*, Wild-type embryo injected with anti-sense RNA. Note unabsorbed yolk (arrows) typical of *sna* alleles²⁶.

Methods. RNA was synthesized from pcSB and pcSBI using the T7 promoter. Autoradiography of labelled RNA produced in parallel reactions showed $\geq 90\%$ of the synthesized RNA to be full-length. Unlabelled RNA was hydrolysed to a mean length of 100–300 bases. RNA for injection was dissolved in 0.1 mM sodium phosphate, pH 6.8, 5 mM KCl at 10 μ g ml⁻¹. Sense or anti-sense RNA (≈ 1 nl) was injected into the mid- to posterior abdomen of wild-type embryos (20–60 min after egg laying). In an additional control series, embryos were injected with an identical volume of buffer. Embryos were scored after 36 h.

alterations in the restriction digest pattern of five alleles (3 strong and 2 weak), when compared with that of wild-type DNA (data not shown). In all cases, these changes mapped to a small region between positions +17.4 and +20 kb (Fig. 1). The modifications observed for *sna*^{RY1}, the strongest mutant allele, result from a 1.6 kb deletion of DNA in this region. Restriction fragments from the region +8 to +32 covered by the recombinants λ 215 and λ 308 (Fig. 1), used as probes on Northern blots of total and poly(A)⁺ RNA from various stages of embryonic development, revealed two transcripts. One of these of 1.7 kb exhibits a developmental profile compatible with that of early zygotic gene expression (inset Fig. 1) and is located precisely in the region where the five *sna* allele breakpoints were mapped. As it was the only embryonic transcript identified in this region, we concluded that this is the *sna* transcript.

To isolate the corresponding cDNA clone, a *Sal*I fragment covering the region +14.6 to +21.4 was used as a hybridization probe to screen an Oregon R 3–12 h embryonic cDNA library¹⁴. A single class of cDNA clones was isolated, the longest of ~ 1.7 kb. Restriction mapping of this 1.7 kb cDNA, subcloned in both orientations into Bluescribe M13⁻ (Vector Cloning Systems) to give pcSB and pcSBI, resulted in its localization to the region +17.7 to +19.4 (Fig. 1). The direction of transcription as determined by annealing single-strand cDNA probes to total embryonic RNA, is proximal to distal with respect to the centromere (Fig. 1).

In order to obtain biological proof that this is indeed the *sna* cDNA, anti-sense RNA was injected into early wild-type embryos. This experimental approach has been used in *Drosophila* to define the segmentation gene *Krüppel*¹⁵ and to

Mutants at the *sna* locus are zygotic embryonic lethals that affect dorsal-ventral patterning. The strongest mutant expression results in abnormal development of the laterally derived ectoderm as well as the ventrally derived mesoderm, whereas in the weaker alleles only ventral structures are affected⁴. The locus maps in the cytological position 35D 1.2 on the left arm of chromosome 2 (ref. 4). Molecular studies of this region started with the *Scutoid* (*Sco*) mutant, in which three genes of the *Adh* region (*noc-osp-Adh*) at 35B have been exchanged with six loci at 35D, immediately distal to *sna*, such that *noc* is now adjacent to *sna* (ref. 12). A DNA fragment from the *noc* locus obtained during a chromosomal walk in the *Adh* region¹³ was used as a probe to screen a *Scutoid* genomic bank. A 3.2 kilobase (kb) *Sal*I fragment of *sna* region DNA was isolated from a fusion recombinant and used for screening an EMBL4 genomic bank of Oregon R DNA for initiating a chromosomal walk of ~ 90 kb (a full report of the walk will be published elsewhere). To localize *sna* within the cloned DNA, genomic DNA from *sna* mutant stocks (3 X-ray- and 12 ethyl methane sulphonate (EMS)-induced) and from wild-type stocks was digested with restriction enzymes and subsequently probed with phage recombinants derived from the walk. Southern blot analysis showed

CCCCCCCCCCCCGATGACCTCAGCTCTGTTGAGAA

44 CGCAACCCGCTGTATACGATCCGAACTATATACTGGCTCTCGATCGCGATCTCCGATTACCCATCTCGATCAGTACCGGAAACCTAACTAATACACACATCAAAA
 164 ATG GCC GCC AAC TAC AAA AGC TGC CCG CTA AAG AAG CGC CCC ATT GTC TTC GTG GAG GAG CGT CTG CCA CAA AGC GAG GCC TTG GCC CTG
 Met Ala Ala Asn Tyr Lys Ser Cys Pro Leu Lys Lys Arg Pro Ile Val Phe Val Glu Glu Arg Leu Pro Gln Thr Glu Ala Leu Ala Leu 30

254 ACC AAG GAC TCA CAG TTT GCC CAG GAT CAG CCG CAG GAT CTA TCC CTG AAA CGG GGT CGC GAC GAG GAG ACC CAG GAT TAT CAG CAG CCG
 Thr Lys Asp Ser Gln Phe Ala Gln Asp Gln Pro Gln Asp Leu Ser Leu Lys Arg Gly Arg Asp Glu Glu Thr Gln Asp Tyr Gln Gln Pro 60

344 GAA CCG AAA CGT GAT TAT GTG CTG [AAC CTT TCA] AAA ACA CCG GAA AGG AAC TCC AGC TCT AGC TCC AAC TCC TGC CTG CTG TCG CCG CCA
 Glu Pro Lys Arg Asp Tyr Val Leu [Asn Leu Ser] Lys Thr Pro Glu Arg Asn Ser Ser Ser Ser Ser Asn Ser Cys Leu Leu Ser Pro Pro 90

434 GTG GAG ACC CAG GAT TAT TTG CCC ACC GAA ATC CAT ATG CGT GGA CTA AGC GGA ACA ACG GGA TAT ACC ACC GCC ACC CCC ACC ACG
 Val Glu Thr Gln Asp Tyr Leu Pro Thr Glu Ile His Met Arg Gly Leu Thr Ala Gly Thr Thr Gly Tyr Thr Thr Ala Thr Pro Thr Thr 120

524 ATT AAT CCC TTC CAG TCC GCC TTT GTG ATG GCC GCG GGT TGC AAT CCG ATC TCT GCC CTG TGG AGC AGC TAT CAG CCC CAT CTG GCC GCC
 Ile Asn Pro Phe Gln Ser Ala Phe Val Met Ala Ala Gly Cys Asn Pro Ile Ser Ala Leu Trp Ser Tyr Gln Pro His Leu Ala Ala 150

614 TTC CCC TCG CCA GCC AGC TCG ATG GCC TCG CCC CAG TCG GTC TAC AGC TAC CAG CAG ATG ACG CCG CCC TCC ACC CCC GGA TCC GAT CTG
 Phe Pro Ser Pro Ala Ser Ser Met Ala Ser Pro Gln Ser Val Tyr Ser Tyr Gln Gln Met Thr Pro Pro Ser Ser Pro Gly Ser Asp Leu 180

704 GAA ACC GGT TCG GAG CCA GAG GAT CTT TCA GTG CGA AAT GAC ATC CCA CTG CCG GCT CTG TTT CAC CTT TGC GAT GAG GCC AAG TCC AGC
 Glu Thr Gly Ser Glu Pro Glu Asp Leu Ser Val Arg Asn Asp Ile Pro Leu Pro Ala Leu Phe His Leu Phe Asp Glu Ala Lys Ser Ser 210

794 AGC AGC GGT GCC AGT GTA AGC AGC TCA TCG GGA TAT TCC TAC ACT CCG GCC ATG AGT GCC TCG TCG GCG AGT GGT GCC GCC AAT CAT GCC
 Ser Ser Gly Ala Ser Val Ser 240

884 AAA AAC TAC CCG [TTC AAG TGC GAC GAG TGC CAG AAA ATG TAC TCC ACC TCA ATG GGC CTG TCC AAG CAC CGT CAG TTC CAC TGC CCG GCA
 Lys Asn Tyr Arg Phe Lys Cys Asp Glu Cys Gln Lys Met Tyr Ser Thr Ser Met Gly Leu Ser Lys His Arg Gln Phe His Cys Pro Ala 270

974 GCC GAG TGT AAT CAG GAG AAG AAG ACC CAC TCC TGC GAG GAG TGT GGA AAG CTG TAC ACC ACC ATT GGA CCC CTC AAG ATG CAC ATC CGC
 Ala Glu Cys Asn Gln Glu Lys Thr His Ser Cys Glu Glu Cys Gly Lys Leu Tyr Thr Thr Ile Gly Ala Leu Lys Met His Ile Arg 300

1064 ACC CAC ACT CTG CCC TGC AAG TGC CCC ATT TGC GGC AAG GCC TTC TCT CGA CCC TGG CTG CTC CAG GGA CAC ATC CGC ACC CAC ACT GGA
 Thr His Thr Leu Pro Cys Lys Cys Pro Ile Cys Gly Lys Ala Phe Ser Arg Pro Trp Leu Leu Gln Gly His Ile Arg Thr His Thr Gly 330

1154 GAG AAG CCT TTC CAG TGC CCC GAC TGC CCA CCA TCC TTT GCC GAC CGC TCG AAC CTG CGA GGT CAT CAG CAC ACC CAC GTG GAC GTC AAG
 Glu Lys Pro Phe Gln Cys Pro Asp Cys Pro Arg Ser Phe Ala Asp Arg Ser Asn Leu Arg Ala His Gln Gln Thr His Val Asp Val Lys 360

1244 AAG TAC GCC TGC CAG GTG TGC CAC AAA TCT TTC TCC AGG ATG TCG CTT CTG AAC AAG CAC TCC AGC TCC AAC TCC ACC ATC ACT ATT GCG
 Lys Tyr Ala Cys Gln Val Cys His Lys Ser Phe Ser Arg Met Ser Leu Leu Asn Lys Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser 390

1334 TAGGATTCCTCGCATATGAATCCCTTACGAGGACACAATTAACCTAAGCAACTCACATTCGCTGGCGAAGACCAACAAACAGACATACTAATATAGCTTGTAAGTCGATCG

1454 GTTTGGTGCTACTATTACTATATTTATTTTCAGCTATAGTCTAAGTTTAAAGTTCATGTATATTCCTTGTGTCACACGAGCAGATTATACCAATCTATTCTTTAATTAATATATAC

1574 CCTATTATGTATTTCTTACTTACTGTTAAGCCAACTGTTGTTAAGATTATTCACCCCTTAAGAAAGAAATTAATATTTTATAAAAAAACAACACACGAAAAA

Fig. 3 Nucleotide sequence of the *sna* cDNA clone pcSB and the deduced amino-acid sequence of the encoded protein. The ATG initiation codon of the long open-reading frame is indicated with an asterisk and an upstream in-frame termination codon is overlined. A translation termination codon TAG is found at nucleotide 1,334. Two putative polyadenylation signals are underlined. The finger region is boxed and a potential glycosylation site¹⁹ is shown in brackets. Dashed lines indicate a potential recognition sequence for tyrosine protein kinase²⁰. **Methods.** The sequence was determined on both strands by the dideoxy chain termination method²⁷. Overlapping DNA fragments for sequencing were generated by limited DNAase I cleavage of pcSB and pcSBI (ref. 28).

identify the *wingless* gene¹⁶. Anti-sense RNA will prevent specific gene expression by hybridizing to the endogenous (sense) transcripts, thus blocking translation¹⁷. This block should result in a phenotype comparable to that shown by mutant alleles (Fig. 2a). Sense and anti-sense RNA, synthesised from pcSB and pcSBI respectively, were injected into syncytial blastoderm wild-type embryos. The injection of sense RNA had no specific effect on the embryonic phenotype. More than 60% of the viable embryos injected with anti-sense RNA showed a *sna* phenotype (Table 1). The phenocopies ranged from weak, showing reduced segmental denticle belts in number and/or in width, to intermediate, denticle-free twisted cuticle (Fig. 2b). The high lethality seen in these experiments is apparently not due to the injection of RNA (sense or anti-sense), as similar results were obtained when buffer was injected.

The cDNA is 1,687 nucleotides long with two closely spaced polyadenylation signals, and non-translated 5' and 3' regions. A comparison with the size of the transcript estimated on Northern blots, suggests that the cDNA must be near to full length. A single long open-reading frame (ORF) of 1,170 nucleotides (from nucleotide 164 to nucleotide 1,334) corresponds to a protein of 390 amino-acid residues with a calculated relative molecular mass (M_r) of 43,000 (43K) (Fig. 3). The first ATG of the ORF is preceded by an upstream in-frame termination codon (at nucleotide 137) and is flanked by a sequence which fits the

CAAA^ACATG consensus sequence for *Drosophila* translation initiation¹⁸. There are both a putative glycosylation site¹⁹ and a potential recognition site for tyrosine protein kinase²⁰, in the 5' region. The most striking feature of the protein is the presence of an internal repetitive sequence showing structural homology to the nucleic acid binding finger domains first described for the protein transcription factor TFIIIA from *Xenopus laevis*⁵ and subsequently found in another regulatory protein in yeast⁶, and in presumed regulatory proteins of *Drosophila*⁷⁻¹⁰ and mouse¹¹. These proteins contain tandem repeats of the consensus sequence (Phe, Tyr)-X-Cys-X₂₋₄-Cys-X₃-Phe-X₅-Leu-X₂-His-X₃-His-X₂₋₆, where X is any amino acid²¹. It has been proposed that the conserved Cys and His residues form a metal binding core and that sequences in between these residues fold into finger-like structures to form nucleic-acid binding regions⁵. There would appear to be no constraints as to the total number of finger structures or for their position within the encoded polypeptides of these genes.

At the carboxy-terminal of *Sna*, between residues 245 and 390 (boxed in Fig. 3), there is a tandem array of five putative finger motifs. There are two deviations from the general consensus in that the sequence between fingers one and two is longer and the last His of finger five is replaced by Asp (Fig. 4). In *Sna*, as in TFIIIA, there is a single copy of a junction sequence, Thr-Gly-Glu-Lys-Pro (TGEKP). This sequence, whose function is not yet defined, is highly conserved between the individual fingers of Krüppel, a protein product of a *Drosophila* developmental regulated segmentation gene^{8,9}, and those of the mouse *mkr1* and *mkr2* genes¹¹. Although not closely homologous to the consensus sequence of TFIIIA-like finger proteins, putative finger-like nucleic-acid binding domains are present in a variety of proteins, including the DNA-binding region of steroid and thyroid hormone receptors^{22,23}. The significance of such domains in this family of proteins resides in the fact that they are all regulatory proteins with nucleic-acid binding activity.

Snail thus appears to be a regulatory gene coding for a

Table 1 Injection of *sna* sense and anti-sense RNA into *Drosophila* embryos

	Total	Lethals*	Viable	<i>sna</i> Phenocopies
Buffer	208	160	48	0
Sense RNA	211	156	55	0
Anti-sense RNA	243	192	51	32

* Includes all classes of nonspecific lethals.

	F	K	C	DE	C	QKM	Y	STSMG	L	SK	H	RQF	H	CPAAECNQEKKT	
	H	S	C	EE	C	GKL	Y	TTIGA	L	KM	H	IRT	H	TLP	
SNA	C	K	C	PI	C	GKA	F	SRPWL	L	QG	H	IRT	H	TGEKP	
	F	Q	C	PD	C	PRS	F	ADRSN	L	RA	H	QQT	H	VDVKK	
	Y	A	C	QV	C	HKS	F	SRMSL	L	NK	H	SSS	N	CTITIA	
TFIIIA	Y	F	X	C	XXXX	C	XXX	F	XXXXX	L	XX	H	XXX	H	TGEKP
ADRI	Y	F	X	C	XX	C	XXX	F	XXXXX	L	XX	H	XXX	H	XXXXX
SRY	X	X	C	XX	C	XXX	F	XXXXX	L	XX	H	XXX	H	XXXXXX	
KR	Y	F	X	C	XX	C	XXX	F	XXXXX	L	XX	H	XXX	H	TGEKP
KR-H	Y	F	X	C	XX	C	XXX	F	XXXXX	L	XX	H	XXX	H	TGEKP
HB	Y	F	X	C	XX	C	XXX	X	XXXXX	L	XX	H	XXX	H	XXXXX
								*							
MKR	Y	X	C	XX	C	XXX	F	XXXXX	L	XX	H	XXX	H	TGEKP	
CONSENSUS	Y	F	X	C	x ₂₋₄	C	x ₃	F	x ₅	L	x ₂	H	x ₃	H	x ₂₋₆

Fig. 4 Amino-acid sequence of the Sna 'fingers' domain aligned with the finger motifs consensus sequences of TFIIIA (ref. 5), ADRI (ref. 6), Serendipity (SRY) (ref. 7), Krüppel (KR) (ref. 8), Krüppel H (KR-H) (ref. 9), Hunchback (HB) (ref. 10), mouse MKR1 and MKR2 (MKR) (ref. 11) and the derived consensus sequence²¹. For HB, X* stands for A, T, C or F.

potential DNA-binding protein, which is the first indication of the nature of the possible interaction between the genes involved in dorsal-ventral pattern formation: the Sna protein could modulate the activity of an individual gene or a set of genes involved in the dorsal-ventral developmental pathway by directly interacting with their regulatory sequences. As it is a zygotic-acting gene, *sna* could be either one of the last genes required for establishing the dorsal-ventral pattern, or the first gene directing the next step of the process, namely the cell differentiation which occurs at the beginning of gastrulation. The *sna* phenotype would be compatible with the gene acting positively to induce genes necessary for ventral cell differentiation or negatively to repress genes characteristic of dorsal cells. Several genes involved in dorsal-ventral pattern formation have been cloned^{3,24} and it will now be possible to study the molecular interactions between these genes and their products.

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Related repressor specificity of unrelated phages

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Phage 16-3 of the nitrogen fixing bacterium *Rhizobium meliloti* is a typical temperate phage as are the lambdoid phages of *Escherichia coli*. To date there is no genetic or physical evidence to indicate that 16-3 is related to the lambdoids. The genome of 16-3 is regulated by two repressors which bind to their operators¹⁻⁹ (Fig. 1a). Here we report that one of the 16-3 repressors (the C repressor) recognizes not only its own operator but also the operator of lambdoid phage 434, and that the 434 repressor reciprocally binds to the 16-3 operator. The 434 and 16-3 repressors show little sequence homology, except in the short operator recognition part, known as the helix-turn-helix motif^{10,11}. The structure of the specific 434-repressor-DNA complex has been determined to 3.2 Å resolution by X-ray diffraction analysis¹². Three amino acid residues of the second α -helix of the helix-turn-helix motif make contacts with DNA base-pair edges in the DNA major groove¹²⁻¹⁴. Of these, two, Gln at position 1 of the α -helix and Gln at position 2, are conserved in 16-3 repressor. Furthermore, the putative 16-3 operator is homologous to the consensus structure of 434 operators¹⁵.

On the basis of genetic⁷ and physical^{3,5} evidence it is known that the C repressor region can be excised from the 16-3 chromosome by the restriction enzymes *EcoRI* or *PstI/HindIII*. Investigation of this 1.3 kilobase (kb) long DNA fragment revealed two promoters (Pc and Pr) and one operator (Or) in the vicinity of the repressor gene. In one series of experiments (Fig. 1b) the putative promoters or promoter-operator units were fused to the promoter deficient tetracycline resistance gene (*tet*) carried on plasmid pGA46¹⁶. The repressor gene was cloned into a modified version of the plasmid pBR328¹⁷. These two plasmids were transformed into *E. coli* cells, and tetracycline resistance was expressed when the inserted promoter was active, but not if the repressor donated by the other plasmid blocked the promoter. The results indicated that both *Rhizobium* phage promoters were active in *E. coli* cells and the repressor controlled Pr through Or but did not interfere with its own promoter Pc (Fig. 1c).

The nucleotide sequence of the C regulatory region is shown in Fig. 2. The first three amino acids at the amino terminus of the C repressor have been identified from the purified protein

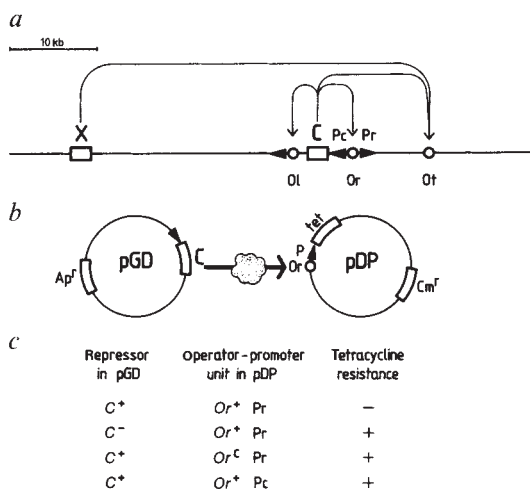


Fig. 1 *a*, Topology of regulatory elements in 16-3. The repressor genes are indicated by open boxes, operators by circles, promoters (with polarity marked) by arrowhead. In refs 8 and 9 Ol, Ot and X are denoted as avirC, avirT and immX, respectively. Top, schematic diagram of regulatory circuits. *b*, Schematic drawing of the compatible and co-selectable plasmid constellation which we used for the identification of C repressor control through the *c*; +, Expression of tetracycline resistance; —, no expression (at 10 μ g ml⁻¹, 37 °C). C⁺ stands for wild-type repressor allele, C⁻ for *ti3* and *ti4* missense mutant alleles^{7,24}, Or⁺ for wild-type operator, Or^c for Or^c-1 mutant operator derived from 16-3 viril immunity insensitive stock⁹. Longer inserts in pDP-s behaved like S280, that is no sign of operator activity functionally linked to Pc was noted.

Methods. pGD plasmids: the *EcoRI*-N fragment of 16-3 DNA was cloned in a truncated variant of pBR328¹⁷. In this cloning vehicle both the tetracycline and chloramphenicol resistance genes were disrupted (by *Bal31* digestion starting from the *EcoRV* site) but the ampicillin resistance gene (*Ap*^r) and the *EcoRI* cloning site remained intact. pDP plasmids are derivatives of the promoter cloning vehicle pGA46¹⁶ carrying in these experiments a 0.28-kb *Sau3A* fragment (S280, part of *EcoRI*-N) in both orientations. In one orientation, S280 induces Pc promoter activity for the *tet* gene, in the other orientation the Pr. pGD/pDP constellation was retained in *E. coli* HB101 by co-selection for chloramphenicol (*Cm*^r) and *Ap*^r resistance. Expression of tetracycline resistance (*tet* gene activity) was investigated in this construction.

(F. Marincs, unpublished data). The position of one base in the Or operator was determined by sequencing the base change in the Or^c-1 mutant operator. Using this position, the polarity of the nearby promoters and the position of two missense mutations in the repressor gene, we determined the open reading frame (ORF-C) and the amino acid sequence of the C repressor (Fig. 2). An inspection of the DNA sequence around the Or^c-1 mutation and of the C repressor sequence (197 amino acids in length) revealed that: (1) The Or^c-1 mutation is in a sequence of dyad symmetry (designated 'Or' in Figs 2 and 3) and a similar sequence 'Or' lies 2.5 helical turns away. The sequence of 'Or' is identical to the consensus sequence of 434 lambdoid phage's operator, and Or^c-1 in 'Or' is homologous to one of the known Or2 operator mutations in 434¹⁵. (2) The conserved helix-turn-helix motif is suggested to lie near the amino-terminus as deduced by the amino acid sequence. (3) The amino acid sequence of the putative helix-turn-helix motif is 55% homologous to that of 434 (Fig. 3); however no apparent homology (<15%) was recognized in the rest of the protein (identical positions, normalized to 434, were 26/187).

The homology found between the helix-turn-helix motifs proved to be biologically significant in both directions: the 16-3 C repressor acted on the 434 operator and the 16-3 Or operator bound the 434 repressor (Table 1). The active 16-3 repressor gene interfered with 434 growth in *E. coli*: at high expression of the 16-3 C repressor, clear plaque-forming 434 CI mutants

Table 1 Interface of 434 and 16-3 regulatory elements

16-3 Element in <i>E. coli</i>			
Plaque phenotype of imm434 strains*			
C repressor expressivity [†]	C ⁺ O ⁺ (G274)	C ⁻ O ⁺	O ^c
None	Turbid	Clear	Clear
Medium	Strong turbid	Turbidish	—
High	Strong trubic	Faint turbid	Clear
Operator (O ⁺) copies‡			
	C ⁺ O ⁺ (G274)	(G907)	
None	Turbid	Strong turbid	
≤10	Clear	Clearish	
~100	—	Clear	

* The phages imm4 and imm21 do not show phenotypic variations. C⁺ stands for the wild-type repressor allele of 434, C⁻ for CI mutant allele, O⁺ for wild-type operators of 434, O^c for mutant operators. C⁺O⁺ strains were NIH (Bethesda, USA) stocks G907 and G274; C⁻ clear mutants were isolated (three independent stocks) from G907; NIH stock G908 virulent mutant served for O^c. O⁺ phages formed 20% fewer plaques (EOP ~0.8) at high induction of 16-3 repressor.

† To assess C repressor expressivity, the C repressor gene of 16-3 (in a *PstI*-*Sma* fragment) was inserted downstream the *tac* promoter of plasmid pKK223-3 at the *PstI* site (*Sma* is an isoschizomer of *Nsi* cuts *PstI* compatible sticky ends at sequence ATGCAT). This plasmid produces a shorter variant of 16-3 C repressor (*Sma* shortens the ORF-C by 8 triplets at the N terminus, see Fig. 2). The expression the C repressor gene was induced by IPTG. In superrepressive *LacR* strain (*E. coli* JM107) medium induction corresponds to an IPTG concentration of 0.5-1 mM, high to 3-5 mM; in the standard *LacR* strain (*E. coli* HB101) the corresponding figure for high induction was 0.5-1 mM. In a parallel experiment the complete 16-3 repressor (in a *PstI*-*HindIII* fragment) was cloned in pGA46, where its transcription was initiated by its own natural promoter Pc. In this case the phenotypic variation of 434 strains was similar to that noted in the 'medium' line. Controls (*E. coli* strains without plasmid and with vector plasmid alone) responded as the 'none' line.

‡ The source of 16-3 Or was fragment S280 (see Fig. 1c legend) cloned in pGA46 (copy number ≤10) and in pTZ19U (copy number 70-200), transformed in *E. coli* HB101, JM101 and JM107 strains. Controls ('none' line): *E. coli* strains with vector plasmids alone.

formed faint turbid plaques, while a turbid plaque-forming stock developed stronger turbidity. These phenotypic shifts were reflected by a decrease in plaque number of ~20%. As expected no phenotypic variation was found between the immunity insensitive (operator) mutant of 434, or lambdoid phages other than 434. On the other hand turbid plaque-forming 434 stocks formed clear plaques when the 16-3 Or operator region was present in extra copies. The more the copies of Or supplied, the more the transparent plaque phenotype developed. This phenotypic shift can be explained by the titration of 434 repressor by the 16-3 Or operator cloned in the multicopy plasmid. Hence the lytic pathway of the phage's life cycle is more favourable, analogous to the induction of the chromosomal *gal* operon by cloned *gal* operators^{18,19}.

Studies on the 16-3 C repressor provide some insights both into evolution and the mode of repressor-operator interaction. The cross functioning between the 16-3 C and 434 CI regulatory regions suggests that compatible repressor-operator combinations might be found in other heterologous experimental systems (for instance *R. meliloti* phage genes in *E. coli* cells). We may expect a limited number of specificity classes (one of them is the 434 CI¹³, Cro¹⁵ and 16-3 C 'family') if the total number of amino acids involved in sequence specific contacts is small^{12,20}. The 16-3C/434CI comparison illustrates the significance of residues 1 and 2 (Gln.Gln) of the second α -helix in the helix-turn-helix motif in recognizing the cognate operators since only these two residues are identical in the DNA contacting surface of the α_3 -helices (Fig. 3).

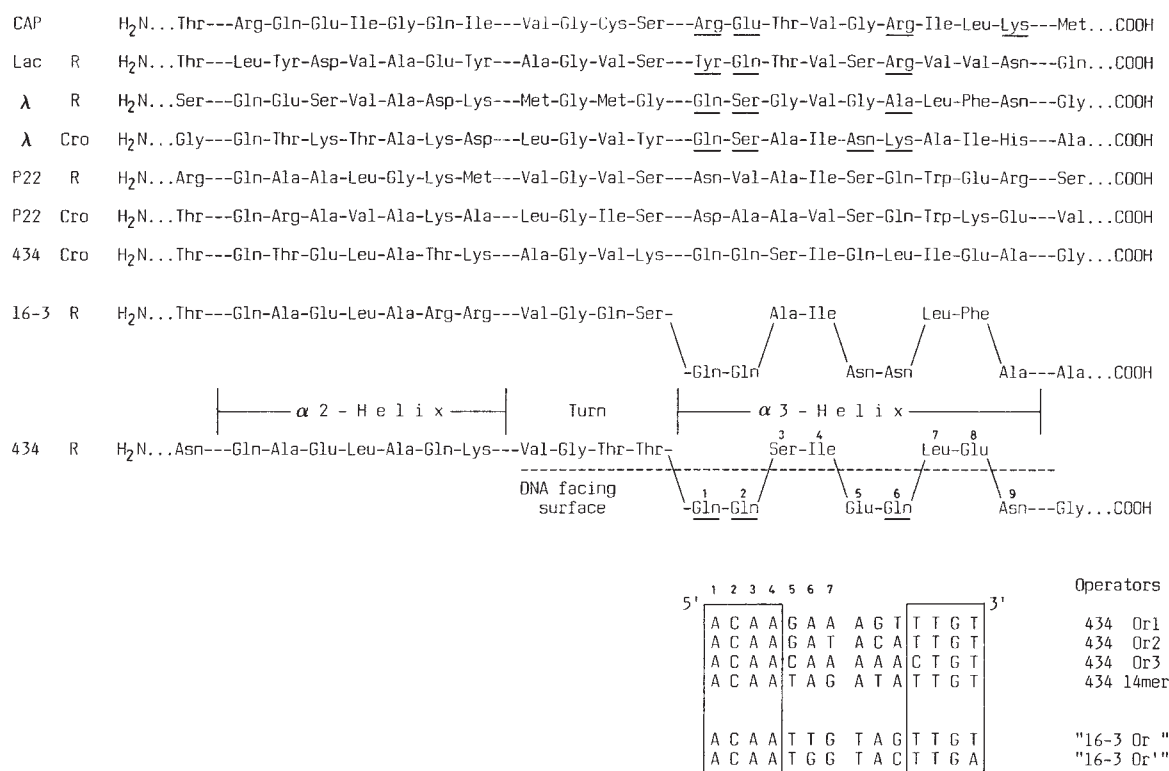


Fig. 2 DNA sequence of the 16-3 C repressor gene and the Pc, Or, Pr control unit. Above, the amino-acid sequence of C (ORF-C). The sequence of the first three amino acids at the amino terminus is His-Lys-Gly (determined in the purified repressor protein by F. Marincs, data not shown). Helix-turn-helix motif and 'Or' putative operator are boxed. Pr and Pc refer to promoter activities (putative -10 and -35 regions are marked). ORF-R denotes an open reading frame of 129 triplets. ti3, ti4 and Or^c-1 are mutations in the C repressor gene and Or operator. This sequence is a part of the *EcoRI*-N and *PstI*57-HindIII60 segment of the 16-3 chromosome⁵ (Pc polarity pointing toward the *PstI* site).

Methods. We used standard cloning procedures²⁵, M13 mp cloning system^{26,27} and the chain terminating method for sequencing²⁸.

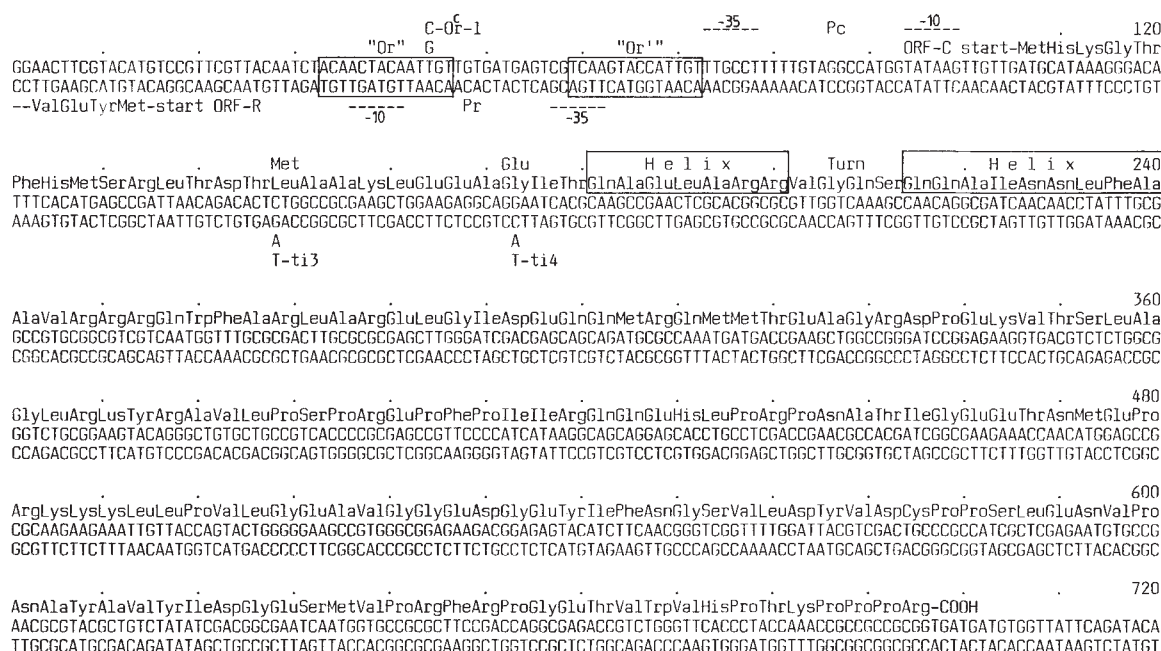


Fig. 3 The operator recognition motif of 434 CI (434R) 16-3 C (16-3R) and several other regulatory proteins¹⁰. Residues that have been proposed to make direct contacts with DNA base-pair edges are underlined (for CAP see refs 20, 23, 29; *LacR* ref. 30; λR and *cro* ref. 31; 434R ref. 12). The DNA sequence of 434 operators and the putative Or operator of 16-3 are also shown. Because in the 16-3 Or operator only one base-pair has been defined by mutation (Or^c-1, Fig. 2), the cross reactivity between 434 and 16-3 repressors and operators (Table 1) is crucial to identifying the 16-3 'Or' site.

When comparing the related binding specificities of the 434 and the 16-3 repressors we see that the Asn residue at position 6 in the operator recognition helix of the 16-3 repressor poses some difficulties in applying the most recent model of 434 repressor-434 14-mer operator interaction^{12,14,21} to 16-3. The corresponding side chain at position 6 in the 434 repressor is longer, a Gln which contacts the operator at base pairs 4 and 5 (see Fig. 3, 14-mer, base pairs 4 A/T and 5 T/A). In the case of 16-3 this Asn/Gln difference might create a wide gap between the recognition α -helix (at position 6) and the operator bases, placing the interacting atomic groups too far from each other. However, coordinated changes in the conformation of the operator with a slight modification of the angle of the recognition helix axis and some rotation of the Asn side chain may narrow the gap between this amino acid and the bases. (For 16-3 we

suggest, for example, a sharper bending of the DNA axis with more local twist at operator base pairs 4 and 5, and a deeper submergence of the α_3 -helix toward the carboxy terminus than those determined for 434¹².) According to recent findings²¹⁻²³ such conformational differences are conceivable.

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Adaptive evolution in the stomach lysozymes of foregut fermenters

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The convergent evolution of a fermentative foregut in two groups of mammals offers an opportunity to study adaptive evolution at the protein level. The appearance of this mode of digestion has been accompanied by the recruitment of lysozyme as a bacteriolytic enzyme in the stomach both in the ruminants (for example the cow) and later in the colobine monkeys (for example the langur). The stomach lysozymes of these two groups share some physicochemical and catalytic properties that appear to adapt them for functioning in the stomach fluid^{1,2}. To examine the basis for these shared properties, we sequenced langur stomach lysozyme and compared it to other lysozymes of known sequence. Tree analysis suggests that, after foregut fermentation arose in monkeys, the langur lysozyme gained sequence similarity to cow stomach lysozyme and evolved two times faster than the other primate lysozymes. This rapid evolution, coupled with functional and sequence convergence upon cow stomach lysozyme, could imply that positive darwinian selection has driven about 50% of the evolution of langur stomach lysozyme.

The majority of evolutionary changes revealed by comparative studies of proteins and nucleic acids appears to fit the neutral theory of molecular evolution; that is, they could have become fixed by random drift of selectively neutral or nearly neutral mutations rather than by positive darwinian selection³. For this

reason, biochemical adaptation has been difficult to demonstrate convincingly at the sequence level. Important adaptive changes may involve only a few of all evolutionary events⁴⁻⁷ and may thus be masked by the preponderance of neutral replacements⁴.

The present study illustrates an approach that minimizes such masking. Here we examine two lineages along which the same new function has arisen independently and compare them with lineages that retain the old function. The old function of lysozyme, evident in many mammals, is to fight invading bacteria^{8,9}. Thus, many mammals—humans and rats included—have moderate to high levels of lysozyme in secretions like tears and saliva, as well as in white blood cells and tissue macrophages⁹. The new function, evident in ruminants and colobine monkeys, is to digest bacteria that pass from the fermentative foregut into the true stomach¹.

The colobine monkey studied here is the hanuman langur (*Presbytis entellus*), which has a large fermentation chamber followed by a tubular stomach^{10,11}, the anterior lining of which contains high levels (about 1 mg per g of tissue) of lysozyme *c*^{1,2}. Saliva, serum and most non-stomach tissues of the langur contain very low levels (about 0.02 mg per g or less) of lysozyme². A similar distribution is seen in cows and other ruminants^{1,12-14}. Thus, the major selection pressure on these cow and langur enzymes may be to act in the stomach fluid.

Langur and cow stomach lysozymes have two characteristics that are consistent with functioning in the stomach fluid, which is acidic and contains pepsin. At physiological ionic strengths, they are most active as catalysts at low pH, in contrast to most conventional lysozymes whose activity profiles are broader and more basic^{1,2}. In addition, they are unusually resistant to breakdown by pepsin¹.

The amino-acid sequence of langur stomach lysozyme was determined and compared to cow and other mammalian lysozymes of the *c* class (Fig. 1). It is most similar in size (130 amino acids) and overall sequence to the other primate lysozymes, with 14 differences from baboon (an Old World monkey with a simple stomach¹¹) and 18 differences from human lysozymes (Table 1).

If these mammalian lysozymes had evolved in a conventional, predominantly divergent manner¹⁵, then we would expect the

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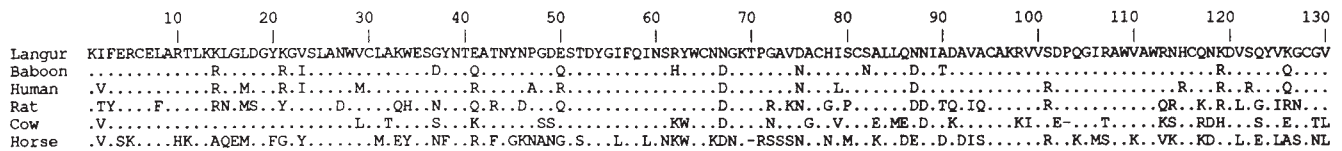


Fig. 1 Amino-acid sequence of langur stomach lysozyme shown in the single letter code and compared to five other lysozyme c sequences from placental mammals. Only differences from the langur sequence are shown for the other sequences, with identities indicated by a dot. An amino-acid deletion is indicated by a dash (-). Sequences shown are from baboon (*Papio cynocephalus*), human (*Homo sapiens*), rat (*Rattus norvegicus*)⁹, cow (*Bos taurus*)^{9,32}, and horse (*Equus caballus*)³³ lysozymes.

Methods. Lysozyme was purified from langur stomach by ion-exchange chromatography (CM52, Whatman) and gel filtration (Sephadex G-50), reduced with dithiothreitol, and the cysteines carboxymethylated with iodoacetamide². Its amino-acid sequence was determined by automated Edman degradation³⁴ on an Applied Biosystems Model 470A gas-phase protein microsequencer. The phenylthiohydantoin amino acids were separated by high-performance liquid chromatography (HPLC; Beckman) on an IBM Cyano analytical column³⁵. The first 60 residues were sequenced from the whole molecule. Residues 51 to 106 were identified from the 9-kilodalton fragment (residues 51 to 130) prepared by complete digestion of the molecule with glutamate-specific protease from *Staphylococcus aureus* strain V8 (Pierce)³⁶. Mild acid hydrolysis yielded a fragment bearing residues 103 to 130 from which the carboxy-terminal sequence was determined². The lysozyme was chemically cleaved³⁷ between Asn 67 and Gly 68, and the two fragments separated by HPLC. The fragment starting from Gly 68 was sequenced past residue 103. To confirm² the sequence of the C-terminal region, this same fragment was chemically cleaved after the tryptophans³⁸, the resulting fragments separated by HPLC, and the fragment bearing residues 113 to 130 sequenced.

parsimony tree built from them to match the branching order of the species (Fig. 2, tree A). Surprisingly, however, the tree that places cow stomach lysozyme with the langur enzyme (Fig. 2, tree B) is as parsimonious as the biological tree. Based on experience with divergently evolving proteins, such as haemoglobins¹⁶, we expected tree B to require at least five more amino-acid replacements than tree A. The unexpected association of cow and langur lysozymes led us to consider three possible evolutionary explanations—horizontal transfer, gene duplication and homoplasy.

Horizontal transfer. The stomach lysozyme gene could have been transferred between an ancestor of the langur and an ancestor of the cow. This mechanism has testable consequences. Recently transferred genes should be nearly identical in overall sequence; yet, the langur and cow lysozymes differ at 32 positions (Table 1). The logical direction for such a selective transfer would be from a ruminant to the ancestral colobine monkey, since foregut fermentation arose more recently along the Old World monkey lineage (about 15–20 million years ago^{17,18}) than along the ruminant lineage (about 55 million years ago¹). However, tree B (Fig. 2) suggests that if such a transfer did occur it was from the monkey to the ruminant because the cow

lysozyme resides in the primate part of the tree. Furthermore, if the cow gene was indeed acquired from a monkey, it should branch with the primates even when the langur sequence is omitted (Table 2, alternative tree D). This is not the case. When the langur sequence is omitted, the most parsimonious tree (Table 2, biological tree C) places cow lysozyme near horse lysozyme as in tree A of Fig. 2. Thus, horizontal transfer is unlikely to be the explanation.

Gene duplication. Suppose, for example, that there are two lysozyme genes, X and Y, resulting from a duplication predating the divergence of primates and ruminants and that the langur and cow stomach lysozymes are the products of the X gene while the other mammalian lysozymes could be the products of the Y gene. If so, langur lysozyme should not only differ greatly in sequence from the other primate lysozymes but also should lie on a lineage that branched off before the Y lysozymes diverged from one another. Instead, langur lysozyme is most similar in overall sequence to baboon lysozyme (Table 1) and is also genealogically close to it in both trees A and B (Fig. 2). In addition, when the cow sequence is omitted, the most parsimonious tree for the five remaining lysozymes (Table 2, tree E) now has the topology of the biological tree. But, if the above

Table 1 Pairwise comparisons of lysozyme sequences

	Species compared	Amino-acid differences					
		La	Ba	Hu	Ra	Co	Ho
Uniquely shared residues	Langur	—	14	18	38	32	65
	Baboon	0	—	14	33	39	65
	Human	0	1	—	37	41	64
	Rat	0	1	0	—	55	64
	Cow	4	0	0	0	—	71
	Horse	0	0	0	0	1	—

For each pair of lysozymes in Fig. 1, the number of amino-acid differences appears above the diagonal; each deletion is counted as one difference. Below the diagonal is the number of positions at which each pair of lysozymes shares an amino acid that is found in no other vertebrate lysozyme of known sequence (excluding other ruminant and colobine stomach lysozymes). The additional lysozymes used here are complete sequences from echidna (H. McKenzie, personal communication), pig (J. Jollès and P. Jollès, personal communication), and numerous birds^{9,28}, as well as partial sequences from tortoise⁹, dog²⁹, goat tears (J. Jollès and P. Jollès, personal communication), and mouse macrophages and intestines³⁰. The number of uniquely shared residues between cow and langur is significantly greater than average (χ^2 test, $P > 0.95$). (The high divergence of horse lysozyme from these other mammalian lysozymes might be due either to accelerated evolution along the horse lineage or expression of a duplicate lysozyme gene².)

Table 2 Comparison of trees for lysozyme

Kind of tree	Minimum no. of amino-acid replacements	No. of wins
Six species		
A Biological	174	11
B Alternative	174	12
Langur omitted		
C Biological	163	13
D Alternative	169	8
Cow omitted		
E Biological	137	11
F Alternative	145	3

The term 'biological' refers to the tree for the species compared; 'alternative' refers to the other possible trees discussed in the text. Biological tree A is shown in Fig. 2; biological trees C and E would look like tree A if the langur (tree C) or cow (tree E) lineages were omitted. Trees C and E were the shortest ones found by the computer program PHYLIP³¹ for the five sequences. Alternative tree D has the branching order that would result if the cow lineage were attached at node L (instead of node C) and the langur lineage omitted from tree A. Conversely, tree F would result if the langur lineage were attached at node C (instead of node L) and the cow lineage omitted from tree A. 'Number of wins' refers to the number of positions in the lysozyme sequence at which one tree requires fewer amino-acid replacements than the other to explain the sequence divergence.

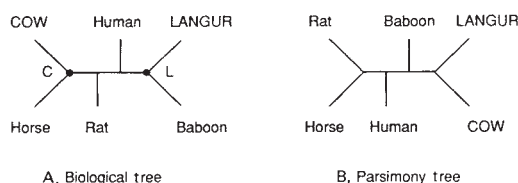


Fig. 2 Shortest, unrooted trees relating mammalian lysozyme sequences. Tree A is the biological tree for the six species, and tree B is an alternative tree for six lysozyme sequences.

Methods. Amino-acid parsimony trees were constructed with a computer program (PROTPARS in the package PHYLIP³¹) that can find the tree requiring the fewest amino-acid replacements. The program requires that any changes be consistent with the genetic code but does not count synonymous substitutions; a change between two amino acids which must pass through an intermediate state (a third amino acid) is counted as two replacements. Each deletion is here counted as one replacement. In this case, there were two shortest trees, each explaining the data with 174 amino-acid changes (see Table 2). This number includes derived replacements, which are the result of shared ancestry and therefore unite sequences according to relationship, as well as homoplasies (parallelisms and convergences), which are the result of independent evolutionary events and therefore do not imply relationship²¹. (Amino-acid parsimony trees were also built by the computer program PAUP³⁹, which allows any amino acid to change into another without regard for the genetic code. This program found trees that unite cow and langur to be the shortest². The ruminant stomach and colobine monkey lysozymes were found to branch together even when other complete and partial sequences listed in Table 1 were included in the trees.) Nodes C and L are explained in Table 2.

gene duplication model were correct, an alternative tree (Table 2, tree F) would have been favoured. The duplication model further predicts that within a given primate both *X* and *Y* lysozymes may be present; however, tissue surveys done with electrophoretic and immunological methods give no evidence of two distinctly related types of lysozyme within humans or monkeys^{1,2}. All evidence to date suggests that these three primate lysozymes are the products of equivalent (orthologous) genes. Thus, gene duplication is unlikely to explain the link between cow and langur stomach lysozymes.

Convergent or parallel evolution (homoplasy). Sequences can also be similar because of independent acquisition of identical amino acids through parallel or convergent replacements, a phenomenon known as homoplasy¹⁹⁻²¹. If the incidence of homoplasy between two distantly related sequences is unusually high, then a parsimony tree is likely to link them^{15,22}. To test for homoplasy in the lysozyme sequences we followed the recommendation of Sneath and Sokal²¹: accordingly, the amino-acid replacements were assigned in the simplest way along the lineages of the biological tree (Fig. 2, tree A). As illustrated in Fig. 3, five homoplastic replacements (producing Lys 14, Lys 21, Glu 50, Asp 75 and Asn 87 in cow and langur lysozymes) were found to occur along the cow and langur lineages. This number is significantly greater (χ^2 test, $P > 0.95$) than the number (0 or 1) of homoplastic replacements found to occur along the other pairs of lineages². Therefore, these five amino-acid replacements are unlikely to have occurred by chance (that is, neutral drift) alone. In addition, two replacements (producing Leu 17 and Ser 101) appear to have occurred in parallel along the lineage leading to cow and along the lineage leading to the Old World monkeys (baboon and langur). Four of the resulting amino acids (Lys 21, Glu 50, Asp 75 and Asn 87) are found only in colobine and ruminant stomach lysozymes (Table 1). Thus, homoplasy is the most plausible explanation for tree B (Fig. 2), since it can account for both the significant number of uniquely shared amino acids and the relatively large sequence difference between langur and cow stomach lysozymes (Table 1).

About half of the replacements which occurred on the langur lysozyme lineage after it diverged from that of the baboon made

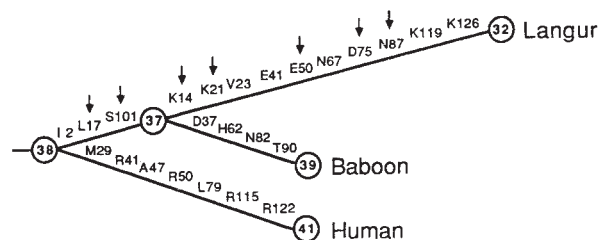


Fig. 3 Apportioning of amino-acid replacements on the lineages leading to primate lysozymes. Branch lengths are proportional to the number of replacements. The resulting amino acids are written in the single letter code followed by numbers indicating their positions in the sequence. The seven arrows point to amino acids which arose independently along the lineages leading to cow and langur lysozymes. (The two homoplastic replacements along the lineage leading to baboon and langur are consistent with the hypothesis^{1,18} that the ancestral Old World monkey was a foregut fermenter.) There were no homoplastic replacements with the cow lineage along the baboon and human lineages after these two primate lineages branched from that leading to langur. In the circles are the numbers of amino-acid differences from cow stomach lysozyme (see Table 1). Since its time of common ancestry with the human and baboon lineages, the langur lysozyme lineage has gained sequence similarity to the cow enzyme: starting with 38 differences, this lineage has ended up with 32 differences from cow lysozyme. In contrast, the human and baboon lysozyme lineages diverged from the cow lysozyme lineage and have ended up with 41 and 39 differences, respectively, from this enzyme.

Methods. Amino-acid replacements were assigned along the lineages of the biological tree in a manner which requires the fewest amino-acid replacements and the fewest nucleotide substitutions. The additional sequences listed in the legend of Table 1, as well as partial sequences from other ruminant (J. Jollès and P. Jollès, personal communication) and colobine² stomach lysozymes, were used when necessary to decide upon the most parsimonious assignments. (The number of replacements along each lineage is consistent with that found in the shortest distance Wagner tree.) Each set of homoplastic replacements was examined to determine whether the underlying mutational mechanisms might have been parallel or convergent^{2,21}. (An example of parallel replacements would be an Arg to Lys change on both lineages. An example of convergent replacements would be Arg to Lys on one lineage and Thr to Lys on the other.) Lys 14 and Glu 50 may have been the result of convergent replacements, while the others appear to have been in parallel.

the langur sequence more similar to cow lysozyme than was the ancestral monkey enzyme (Fig. 3); that is, langur lysozyme converged in sequence upon cow lysozyme. In contrast, baboon and human lysozymes followed normal divergent paths. So, half or more of the replacements along the langur lysozyme lineage may have been fixed by positive darwinian selection. Consistent with this interpretation, langur lysozyme appears to have evolved about twice as fast as the other primate lysozymes (Fig. 3).

The possibility that sequence convergence has accompanied the convergent regulatory and functional evolution of langur and cow lysozymes was at first surprising. Comparative biochemistry has repeatedly shown that a given function can be fulfilled by a vast array of sequences and had reinforced the notion that sequence evolution, whether dominated by drift or positive selection, is mainly divergent. Lysozyme seemed to be typical in these regards: in their amino-acid sequences, the *c*, *g* and phage classes of lysozymes have diverged greatly and yet have the same bacteriolytic function²³. Furthermore, the well-characterized cases of functional convergence in proteins are not the result of convergent sequence evolution. Haemoglobin and haemocyanin, for example, are functionally convergent but these two types of protein exhibit no sequence similarity. Likewise, subtilisin and the trypsin-like serine proteases independently evolved similar active sites but did so with different primary and tertiary structures²⁴.

Despite the predominantly divergent nature of most sequence evolution, parallel and convergent events often occur; these usually appear to be scattered randomly across species²² and only rarely have functional explanations for specific replacements been proposed²⁵. Moreover, when selection for a new protein function is imposed on bacterial populations in the laboratory, the same way of solving the problem is sometimes encountered²⁶. So, there may be a limited number of ways of converting a conventional mammalian lysozyme into one that would function well in the stomach fluid of a foregut fermenter. To understand why langur lysozyme has gained sequence similarity to cow stomach lysozyme, studies must be made of the functional consequences of the homoplastic replacements identified by our phylogenetic analysis. For evaluation of some of the possible consequences, see ref. 27.

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ERRATUM

Location of the ATPase site of myosin determined by three-dimensional electron microscopy

M. Tokunaga, K. Sutoh, C. Toyoshima & T. Wakabayashi
Nature **329**, 635-638 (1987).

In this letter Fig. 1c was incorrectly printed alongside Fig. 4a and b.

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Notes on Nordic science products

As an accompaniment to this week's feature section on the state of science in Sweden, Finland, Denmark and Norway, Product Review turns to research and industrial products of Nordic origin.

PERSTORP Biolytica, in Lund, Sweden, has a new **recombinant protein G** that binds a broad spectrum of immunoglobulins, with high affinity (*Reader Service No. 100*). The company says protein G is similar to protein A, but binds to a wider range of immunoglobulins from animals of several species. It also binds to specific IgG subclasses, and functions under normal physiological conditions of neutral pH and low ionic strength. Perstorp Biolytica incorporates its protein G into its SelectiSpher-10 columns for high performance liquid affinity chromatography, an agarose gel for gel affinity applications, and a horseradish peroxidase preparation for ELISAs.

Also in Lund, Sweden, MonoCarb AB is putting the finishing touches on its new Skr15 million facilities for large-scale mammalian cell culture. The company plans to use the facilities to produce **monoclonal antibodies** for both diagnostic and therapeutic applications. MonoCarb has targeted the bulk monoclonal antibody market, and will sell its antibodies to diagnostic firms for inclusion in clinical kits (*Reader Service No. 101*). MonoCarb currently sells monoclonals for blood grouping.

In its headquarters in Lund, Sweden BioInvent International AB has used an **in vitro immunization system** for years to produce custom orders of murine monoclonal antibodies from weak immunogenic antigens (*Reader Service No. 102*). Now BioInvent is offering its Vitrotech system to others. BioInvent says the Vitrotech system allows antigen-specific activation of murine B cells in a tissue culture flask, in only five days. The activated B cells can then be used as parental cells in somatic cell hybridization experiments and for the production of monoclonal antibodies. Vitrotech side-steps the

normal regulation of the immune response that occurs *in vivo*, so monoclonal antibodies can be produced against phylogenetically very conserved structures like calmodulin, actin, or histones. Only small amounts of antigen are needed for immunization: BioInvent says successful immunization has been achieved with an antigen concentration of as low as 1 ng ml⁻¹. Each Vitrotech kit includes enough spleen-preparing medium, biological response modifier, control antigen, spleen disintegrator, and lymphokine cocktail for three *in vitro* immunizations.

Pharmacia, in Uppsala, Sweden, has scaled up its FPLC chromatography technique for the separation of biomolecules into a **process development system** — the BioPilot (*Reader Service No. 103*). Pharmacia says the BioPilot system allows companies to move easily from process



The BioPilot process development system from Pharmacia is for scale-up or small-scale production.

development to production-scale separations, because it uses only methods that are applied routinely in industry. It can be used for method scouting and process optimization, up through the small volume production of enough material for clinical trials, to the ultimate purification step at the end of a large-scale production process. The BioPilot uses Pharmacia's MonoBead or Sepharose cross-linked agarose columns for ion exchange chromatography, and its Superose cross-linked agarose columns for gel filtration. The system can be set up automatically to run in a one-pump, one-column, one-monitor arrangement, or up to a three-pump, seven-column, three-monitor configuration.

Tecator AB, in Höganäs, Sweden, has developed a refinement of the Soxhlet extraction process for the **solvent extraction** of samples prior to analysis. Called the Soxtec technique, Tecator's process involves two extraction steps and one

solvent recovery cycle. In the first extraction step, the sample is placed in an extraction thimble and immersed in boiling solvent. In the second step, the thimble is raised above the surface of the solvent, and a continuous flow of freshly condensed solvent is dripped through the thimble to finish the extraction. In the last step, the stopcock valve is closed to enable the recovery of up to 70 per cent of the solvent for reuse. Tecator says the Soxtec technique requires 20 per cent less time than a Soxhlet extraction. Tecator sells two Soxtec systems: the Soxtec HT2 holds one or two samples, and the Soxtec HT3 holds up to six samples (*Reader Service No. 104*).

Bio Ion Nordic AB, in Uppsala, Sweden, has a new tool for peptide and protein chemists — the Bio Ion 20 **plasma desorption mass spectrometer** (*Reader Service No. 105*). The Bio Ion 20 is a time-of-flight mass spectrometer with two detectors. The sample under analysis is deposited on a metal foil, and bombarded with fission fragments from the spontaneous fission of ²⁵²Californium. The fission fragments ionize the sample molecules and cause desorption of the ionized molecules from the foil. The molecular ions are then allowed to drift in an 18-cm field-free tube, and the flight time for each is measured with a time resolution of 1 ns. The instrument converts flight time into a mass spectrum according to a standard equation. Bio Ion Nordic AB says the technique can differentiate proteins of up to 30,000 Daltons by a single amino acid.

Carbohydrates International AB is a one and a half year-old spin-off of the Swedish Sugar Company, located in Malmö, Sweden. Carbohydrates International concentrates on the research and production of **biologically active carbohydrates**, and has divisions dedicated to organic synthesis, bioorganic synthesis and diagnostic applications. The company currently sells a diagnostic kit to detect infections of the human urinary tract by *P. fimbriata* *Escherichia coli* (*Reader Service No. 106*).

Danish products

To help the biotechnology industry conform to the US Food and Drug Administration requirement that all products made through genetic engineering processes be free of hybridizable DNA and RNA, Alfred Benzon AB, in Copenhagen, Denmark, offers an **industrial nuclease** (*Reader Service No. 107*). The



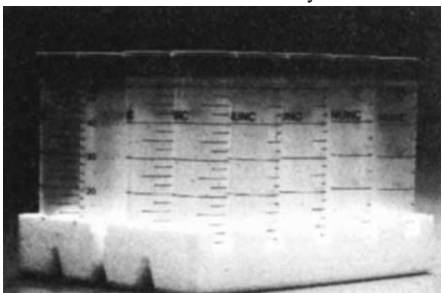
BioInvent's in vitro immunization system activates murine B cells in only five days.

Benzon nuclease is produced by a *Serratia marcescens* gene inserted into *E. coli*, and is collected from the growth medium of the transformed microbe. Alfred Benzon AB says the nuclease is active from pH 6 to pH 10, and degrades large DNA and RNA molecules into oligonucleotides of between 2 and 4 nucleotides. The nuclease is free of proteolytic activity, and remains active in the presence of small amounts of surfactants to degrade DNA or RNA protected by a protein coating. Industrial grade Benzon nuclease is available as a lyophilized white powder with mannitol, with a purity of over 90 per cent.

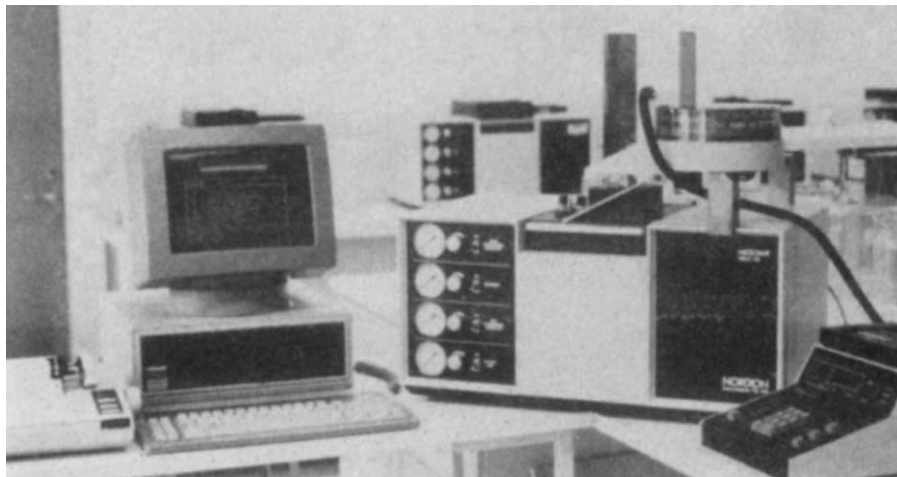
The Dako Corporation, a subsidiary of Dakopatts A/S in Glostrup, Denmark, sells a line of rabbit and mouse antibody Quick Staining Kits for **tissue staining**, based upon labelled avidin-biotin (*Reader Service No. 108*). The newest in the line are kits for staining formalin-fixed, paraffin-embedded tissue samples for prostate-specific antigen, keratins, vimentin, and kappa and lambda light chains. Dako says staining can be performed in less than 15 minutes using the Quick Staining Kits, without the need for extra steps to block non-specific protein binding or lengthy buffer baths. The kits are useful for the study of neoplastic tissues.

FMC BioProducts Europe, near Copenhagen, Denmark, has a low gelling/low melting temperature **agarose**, called InCert (*Reader Service No. 109*). InCert agarose is used to make gel plugs for use in alternating field gel electrophoresis techniques for the separation of large DNA fragments, such as pulsed field gel electrophoresis, orthogonal field alternating gel electrophoresis, field inversion gel electrophoresis, and transverse alternating field electrophoresis. FMC BioProducts says InCert can also stand up to use with restriction endonucleases.

Nunc A/S, in Roskilde, Denmark, has widened its range of products for centrifugation by adding a line of 50 ml **centrifuge tubes** (*Reader Service No. 110*). The tubes are made of polypropylene, and have screw-on blue flat tops. The tubes are graduated at 2.5 ml intervals with easy-to-read blue lines printed 180 degrees around each tube. Nunc says the tubes



Nunc's 50 ml polypropylene centrifuge tubes have easy-to-read graduations.

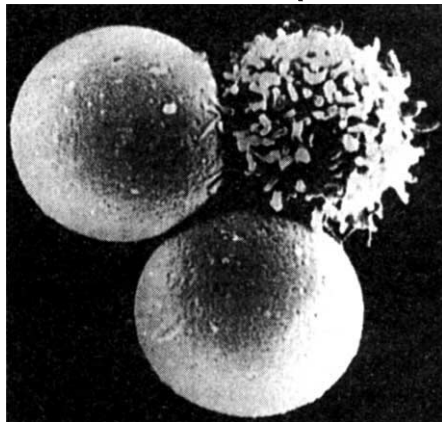


The complete Nordion system for the analysis of over 100 pesticides from one sample.

were designed with the centrifugation of cells in mind, but they are also useful for a variety of storage and transport purposes. The tubes come pre-sterilized by irradiation, and are either mounted in racks or packed in sleeves.

New from Norway

The M-450 Dynabeads from Dynal A/S, in Oslo, Norway, are super-magnetic 4.5 μm **polystyrene beads** for the immunomagnetic separation of cells (*Reader Service No. 111*). The beads have a magnetite core of 26 per cent iron, and a hydrophobic, hydroxyl group-containing polystyrene coating that binds monoclonal antibodies and other proteins. The



Dynal's beads have a great attraction.

Dynabeads are used in conjunction with the Dynal Magnetic Particle Concentrator: once the beads have bound to the target cells, the tube containing the cell culture is exposed to a magnetic field, allowing nontarget cells to be decanted or aspirated off. Dynal says the beads are autoclavable, and are consistent in size with a coefficient of variation of less than 3 per cent.

A high resolution **flow microphotometer** is new from Skatron A/S in Tranby, Norway (*Reader Service No. 112*). Skatron says its flow microphotometer is based on an arc lamp light source instead of the traditional laser, allowing the

measurement of fluorescent markers down to $1.1 \cdot 10^{-17}$ g per cell. The instrument's high resolution makes it suitable for applications such as the preparation of DNA histograms from bacteria, and the study of large viruses and phage. The data system can accommodate up to five parameters, and uses menu-driven software.

Dyno Particles A/S, in Lillestrøm, Norway, has a monosized **polymer sphere calibration kit** for use with electron microscopes, blood-cell analysers, particle analysers or any instrument that requires adjustment for the differentiation of extremely small features (*Reader Service No. 113*). Dyno Particles says the Dynospheres are highly uniform in shape and size, and exhibit a coefficient of variation of less than 2 per cent when analysed using a Coulter Counter. Each Dynospheres kit contains five bottles of 100 mg particles in a 10 ml suspension. The five particle sizes can be chosen by the user from a selection of 0.5, 2, 5, 7, 10, 15 and 20 μm particles.

Finnish finale

Nordion Instruments Oy Ltd, in Helsinki, Finland, has introduced a dedicated gas chromatographic system for the multi-residue **analysis of pesticides** (*Reader Service No. 114*). The Micromat RIM system uses dual channel capillary columns and an updatable pesticide analysis library to provide the capability of screening over 100 compounds from one sample. The system functions using a linear retention index that allows the determination of indices for both rapidly and slowly eluting compounds in a single analysis. Simultaneous analysis in two columns saves time by decreasing the number of confirmatory analyses that are required. The instrument is equipped with an autosampler. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further information, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.